AN ECOLOGICAL STUDY OF BUTTER CLAM (SAXIDOMUS GIGANTEUS) TOXICITY IN SOUTHEAST ALASKA

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AN ECOLOGICAL STUDY OF BUTTER-CLAM (*SAXIDOMUS GIGANTEUS*) TOXICITY IN SOUTHEAST ALASKA

A

THESIS

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ABSTRACT

Butter clams (Saxidomus giganteus) at South and North Porpoise Islands and Pleasant Island, Southeast Alaska, were occasionally found to accumulate significant amounts (higher than the maximum human tolerance) of paralytic shellfish poison (PSP) at any season of the year, and to occasionally lose or regain PSP rapidly between two samplings. The fluctuations of toxicity levels were not similar at all stations and no consistent patterns were observed. The toxicity of only 19 out of 53 samples collected at three high-toxicity stations exceeded the maximum human tolerance level for PSP (1200 MU), and clam samples taken from moderate and low-toxicity stations never exceeded this level. Neither phytoplankton populations nor hydrographic parameters had a consistently significant correlation with toxicity levels; however, fluctuations of phytoplankton numbers demonstrated an inverse relationship with fluctuations of inorganic nutrient concentrations. Dinoflagellate maxima tended to occur at relatively low salinity (22‰ - 29‰) and relatively high temperatures (7°C - 16°C), whereas the diatom numbers did not significantly correlate with salinity or temperature. The three high-toxicity stations were all within Icy Passage; fluctuations of phytoplankton populations and the hydrographic conditions at these stations were similar, yet the fluctuations of toxicity levels were quite dissimilar. A number of possible sources may be responsible for the butter-clam toxicity in Southeast Alaska. However, more studies are needed to define the cause of the butter-clam poisoning problem in Southeast Alaska.

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INTRODUCTION

The first shellfish poisoning in Alaska occurred in 1799 when a group of Aleut hunters from Unalaska and Kodiak stopped at Peril Strait, near Sitka, Alaska, and consumed a dinner of shellfish. According to Petroff (1884) more than 100 men died in less than two hours. In subsequent years numerous shellfish poisoning cases were recorded in Southeast Alaska, several resulted in deaths (Sommer and Meyer, 1937). Twenty-five cases of shellfish toxicity were reported at Porpoise Island (U.S. Public Health, 1962), an area investigated in my study and described in this thesis.

The butter clam, Saxidomus giganteus (Figure 1), one of the most palatable Alaskan species, is plentiful along the coast of Southeast Alaska. It was formerly of commercial importance in Alaska, and a fishery for this mollusc supported a canning industry from 1943 to 1946. In 1946, the U.S. Food and Drug Administration discovered that certain shipments of butter clams from Alaska were very toxic to humans; these shipments were withheld from the market. From this time on commercial shipments of butter clams were continuously monitored for toxicity which ultimately led to the decline and collapse of the canning industry in Southeast Alaska (Magnusson and Carlson, 1951).

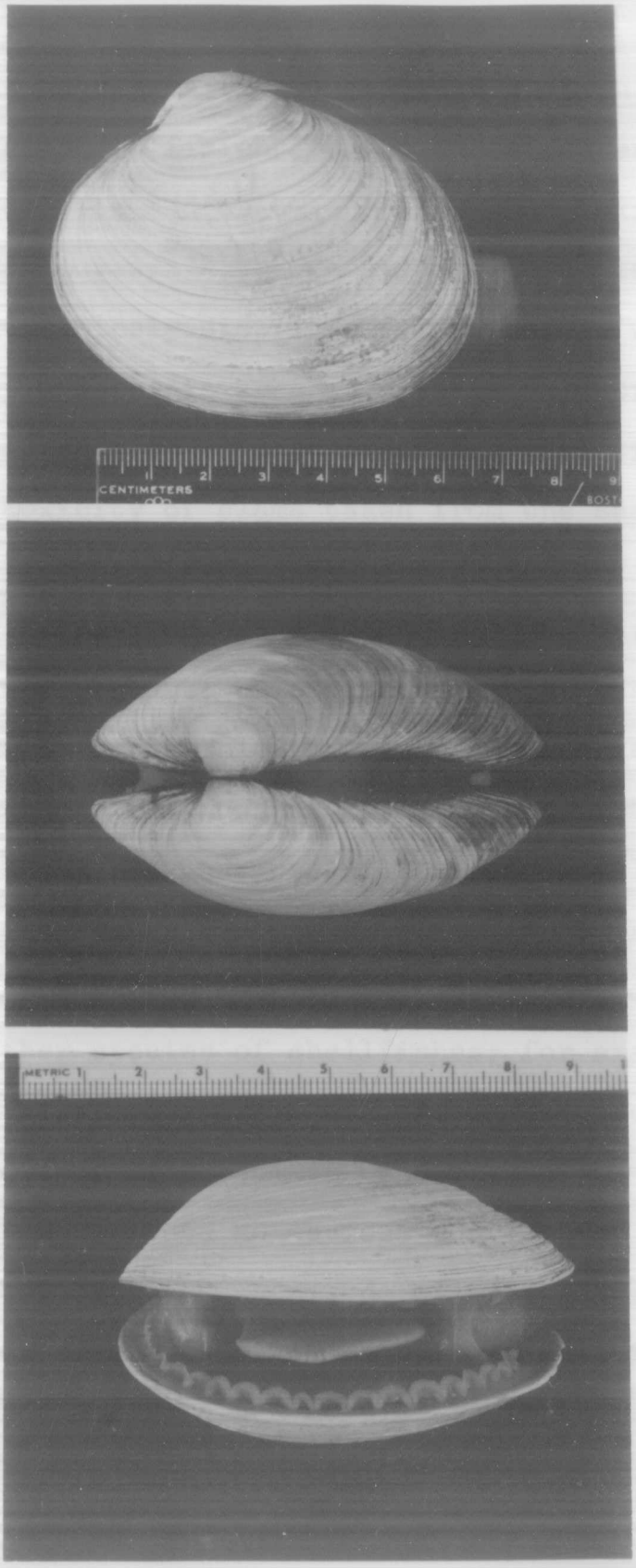


Figure 1. Saxidomus giganteus

Chamber and Magnusson (1950) reported that in many areas clams contained sufficient poison to be dangerous for human consumption and that generally when toxic remained so for several years. They concluded that clams of moderate toxicity were widely distributed and that it was impractical to identify a specific area as either toxic or non-toxic. In addition, they found a considerable variation in the levels of toxicity of clams taken from the same beach. No seasonal patterns of toxicity were found, and the relationships between environmental conditions and levels of toxicity were obscure; this situation made analysis of the data virtually impossible (Magnusson and Carlson, 1951).

Chambers and Magnusson (1950) made a systematic study of the seasonal fluctuations of toxicity levels of butter clams. The tendency for butter clams in Alaska to maintain their toxicity throughout the year differs from the pattern found in other species of shellfishes; for example, California mussels (Mytilus californianus) are only toxic for relatively short periods of time (Sommer et al., 1937). Chamber and Magnusson (1950) found no obvious correlation between degree of toxicity and magnitude of the tides, water temperature, or amount of sunlight. In addition, they found that butter clams retained more toxin in their siphons than in their bodies, although the ratio of toxin levels of siphon to body was never constant. The seasonal variation

observed in clams was restricted to the siphons, whereas the bodies demonstrated no particular seasonal pattern.

Shellfish toxicity is not due to bacteria, parasites, or inorganic salts (Sommer et al., 1937). Smith (1928) found diatoms and dinoflagellates are the main food items for the butter clams. Dinoflagellates of many species, especially of the genus Peridinium, were present in the digestive tract of the butter clam. The finding of plankton in stomach contents of mussels stimulated Sommer et al. (1937) to undertake an investigation of the specific plankton forms used as food by mussels. In this study they found that diatoms and dinoflagellates were the dominant food items. Experimental evidence for the dinoflagellate origin of shellfish poisoning was obtained by Sommer et al. (1937). They showed that nontoxic California mussels became toxic after experimental feeding with a dinoflagellate, Gonyaulax catenella. Mold et al. (1957) ultimately proved that the toxins in clams and mussels were identical in chemical and physical properties. The toxin produced by the dinoflagellate G. catenella in axenic culture was isolated in pure form by Burke et al. (1960). Their study of the chemical, physical and biological properties of the toxin established that it is similar to saxitoxin (the toxin isolated from toxic Alaska butter clams) and to the poison isolated from toxic California mussels. This finding strongly suggests that the toxin in mussels and butter-clams is derived from G. catenella.

RESEARCH PURPOSES

This work was carried out as part of a project with the following goals:

1. A search for correlation of all the major hydrographic parameters with clam toxicity levels.
2. Identification and quantitative analysis of the phytoplankton populations, and a search for correlation with clam toxicity.
3. Isolation and cultivation of representative phytoplankton organisms to test for their toxicity.
4. Detoxification of toxic clams.

This thesis is concerned with the relationships of the hydrographic conditions, phytoplankton populations and clam toxicity levels. The investigation, part of a continuing effort, was carried out on a monthly basis from August 1968 to October 1969, except during the summer. Two field trips were made each month to Porpoise Island and Pleasant Island in June, July and August 1969. The emphasis of the research was on the variation of butter-clam toxicity levels at each clam bed, on the qualitative and quantitative analyses of populations of phytoplankton species in areas near the clam beds, and on the measurement of hydrographic conditions adjacent to the clam beds. The hydrographic variables measured were: inorganic nutrients (i.e. nitrate, nitrite, phosphate and silicate), salinity, water temperature, and water transparency.

STUDY AREAS

The research stations were located in the vicinity of Icy Strait, Southeast Alaska. A map of the study area is presented in Figure 2.

The sites were chosen to include areas with histories of relatively high (stations CT 1-a, CT 1-b, CT 2), moderate (station CT 5), and low toxicity (station CT 7, CT 109, CT 8) (Dr. K. V. Natarajan, personal communication). It was hoped that a comparison of the data from the above three areas might give an indication of cause and effect phenomena related to the toxic butter clam problem.

The location and the beach type of each clam sampling site is described in Table 1.

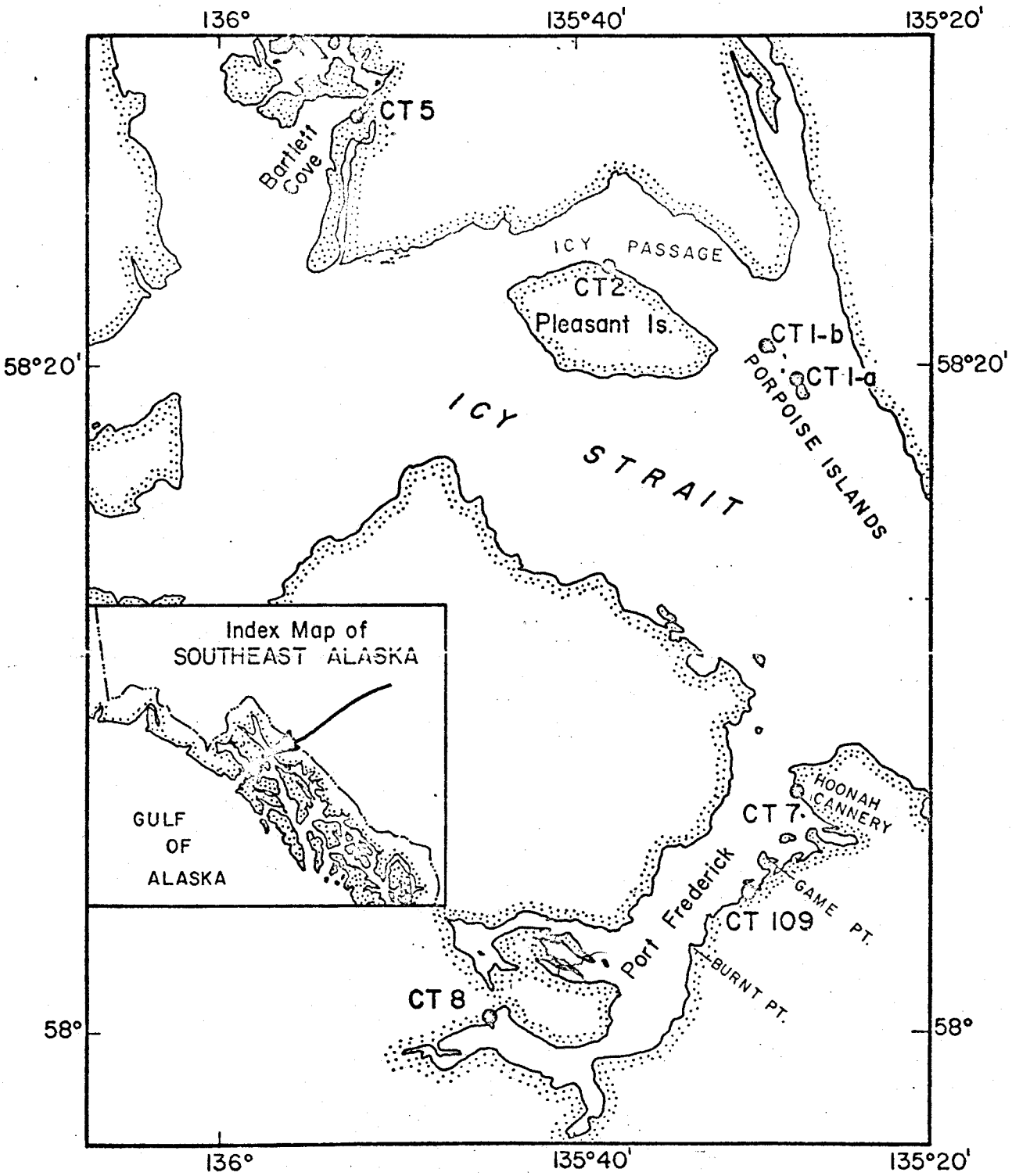


Figure 2. Map of the vicinity of Icy Strait with locations of sampling sites.

Table 1. Location and beach type

STATION NO.	LOCATION	LATITUDE N.
CT 1-a	North corner of S. porpoise Is.	58° 19.6'
CT 1-b	North corner of N. Porpoise Is.	58° 20.6'
CT 2	North side of Pleasant Is. near light house	58° 23.1'
CT 5	Bartlett Cove, Glacier Bay	58° 27.5'
CT 7	Hoonah Cannery, Port Frederick	58° 07.0'
CT 109	Between Game Pt. and Burnt Pt., Port Frederick	58° 04.5'
CT 8	Head of Port Frederick	58° 00.2'

of sampling sites.

LONGITUDE W.	BEACH TYPE AT SAMPLING SITE
135° 27.4'	Mud, gravel, rock
135° 29.0'	Gravel, rock
135° 37.7'	Mud
135° 52.3'	Mud, rock
135° 27.3'	Gravel, rock
135° 30.2	Gravel
135° 43.8'	Mud

METHODS

Biological Sampling Methods

Butter clams from seven stations were monitored for toxicity on a monthly basis. About 5-10 equal sized butter clams were taken from a restricted area at the lowest level of the intertidal zone, and kept in a tray filled with seawater until toxin extraction procedures could be accomplished.

A plankton tow was made at each station adjacent to the beach from which clam samples were taken. A No. 25 plankton net (mesh size 0.064 mm; 200 meshes/in.) with a No. 25 cup was towed for 30 minutes just below the surface. On retrieval, it was essential to rinse the plankton net with seawater to wash the plankton into the cup. Ninety ml of the sample was preserved in a 10 percent formalin solution.

Hydrographic Sampling Methods

Hydrographic stations were established at the same location as the plankton tows. Submarine photometer or Secchi disc readings were taken to estimate the depth of 100 percent, 50 percent, 25 percent and 10 percent light transmission levels. Hydrocasts were made with four 5-liter capacity General Oceanics Nonmetallic Water Sampling Bottles (PVC water sampler) at four depths corresponding to the different light transmission levels. A water sample from each PVC water sampler

was stored in a 250 ml glass bottle for eventual determination of salinity.

For phytoplankton enumeration, the seawater from each PVC water sampler was filtered through an HA 0.45 µm Millipore filter until the pores were completely clogged with phytoplankton; two-four liters were usually required. The Millipore filter with the settled phytoplankton was then scraped with a vinyl policeman into a subsample of 6.25 percent neutralized glutaraldehyde-seawater solution. The subsample volume, 0.3-10 ml, depended on the density of the phytoplankton deposited on the filter. This subsample was then transferred into a screwtopped glass tube. The number of liters of seawater used for filtration and the number of milliliters of subsample preserved were noted for the calculation of phytoplankton density. About 500 ml of filtrate from this filtration procedure was preserved with 2 percent $HgCl_2$ in an acid-washed polyethylene bottle for inorganic nutrient (i.e. nitrate, nitrite, phosphate, and silicate) analysis.

Four salinity samples, four nutrient samples, and four phytoplankton subsamples were collected from each individual station. In addition, water temperatures at each hydrostation was measured by bucket thermometer for surface temperature and by bathythermograph (BT) for temperatures at moderate depths. All hydrographic samples were taken immediately before or after biological sampling.

Extraction of Butter-Clam Toxin

The method used for extracting butter-clam toxin in this research is a modification of that described in Halstead (1965). The procedures used in connection with the extraction are described below.

1. Length, breadth, total weight of clam, and weight of shell were taken for each clam.

2. The outside of the clam was cleaned with fresh water. The shell was opened by cutting the adductor muscles, and rinsed with fresh water to remove sand or other foreign material.

3. The clam meat was washed in running tap water for 5 minutes, and allowed to drain for an additional 5 minutes.

4. The extraction procedure was as follows:
   (1) 100g of meat were weighed and transferred to a Ronson blender with a heating element.

   (2) 100 ml of 0.1 N HCl were then added and blended until the mixture was homogenous. Simultaneously the mixture was heated slowly to boiling and stirred while boiling for exactly five minutes.

   (3) The mixture was poured into a beaker and allowed to cool to room temperature.

   (4) 5N HCl or NaOH was used to adjust the pH of the cooled mixture to 3-4 as determined by narrow range (pH 3.0-5.5) Hydrion pH papers (manufactured by Micro Essential Laboratory, Brooklyn, N.Y.).

   (5) After the adjustment of pH the volume of the mixture was measured and made up to 200 ml with distilled water.

   (6) 30-40 ml of the mixture was centrifuged until a clear supernatant liquid was obtained.

   (7) About 15 ml of the supernatant liquid (the extract-test solution) was collected and refrigerated for the bioassay.

Mouse Bioassay for Butter-Clam Toxicity

Several different methods have been used in the bioassay of paralytic shellfish poison (PSP). A mouse bioassay is the standard method for determination of the amount of PSP in shellfish extract (Halstead, 1965).

1. Mice weighing from 18 to 22 grams were selected. All mice were of the Charles River Mouse Strain.

2. The refrigerated extract-test solution was warmed to room temperature. One ml of extract was then injected intraperitoneally with a 1 ml sterile syringe. The elapsed time of injection to time of death was measured with a stop watch. Death was indicated as the final gasping breath.

3. If the death time was less than 5 minutes, the extract was diluted with distilled water so as to obtain a death time between 5 and 7 minutes. If large dilutions were necessary, the pH was readjusted to 3-4 by addition of 0.1 N HCl.

4. If the death time of one or two mice inoculated with an undiluted supernatant liquid was between 7 and 60 minutes, no concentration adjustment procedure of the supernatant was necessary.

5. Calculation of toxicity
   (1) The death time was determined for at least three mice in order to determine the toxicity of the sample with confidence. From Sommer's table (Appendix A) the number of mouse units (MU) corresponding to the death time was determined.

   (2) A weight correction was made from Sommer's table (Appendix A) for all mice not weighing exactly 20 grams.

   (3) Since the original clam extract was made up to 200 ml, the amount of toxin in mouse units per 100 g of butter clam meat was calculated from the following equation.

$$MU/100\ g = \frac{Do \cdot W \cdot 200}{D}$$

Where Do is the mouse units of the injection from Sommer's table, W is a correction factor for the weight of the mouse, and D is a dilution factor (ratio of the original volume of butter-clam extract to the final volume of solution for the mouse bioassay). The mean from the assay of three mice was taken as the result.

The results of the bioassay were reported in mouse units (MU) per 100 grams of butter clam meat i.e. MU/100 g. A mouse unit is defined as the amount of toxin in 1 ml of an extract which, when injected intraperitoneally, will kill a 20-gram mouse in 15 minutes with symptoms of paralysis and respiratory failure (Prakash, 1967).

Enumeration of Phytoplankton

Phytoplankton cells were counted with a plate model Palmer slide (nannoplankton counting cell) (Palmer and Maloney, 1954) using a Wild compound microscope equipped with a Whipple ocular micrometer. This slide is shallow enough so that a high dry objective (43x - 45x) can be used. The unit consists of a microscope slide with a disc-shaped chamber having a diameter of 17.9 mm, a depth of 0.4 mm, and two narrow channels through the wall. When the disc-chamber was covered with a cover-glass (22 mm^2, No. 1 1/2) it held 0.1 ml of seawater.

The subsample (see Hydrographic Sampling Methods, Page 10) was shaken and transferred by a disposable Pasteur

pipette into one of the two channels on the Palmer slide chamber and a cover glass placed over the chamber. The phytoplankton was identified at 400x to species whenever possible. When qualitative analysis of the phytoplankton was not possible at the time of counting, drawings of the organisms were made for later identification. Each sample was counted on triplicate slides, and 25 fields were counted for each sample.

The results were calculated according to the following procedures:

1. A Whipple field was calibrated at 400x with the aid of a stage micrometer. The area of one field is 0.04 mm^2. The depth of the Palmer slide chamber is 0.4 mm and 25 fields were counted for each slide. The total volume of the 25 fields (V) was obtained as follows:
   $V = 0.04\ mm^2 \times 0.4\ mm \times 25 = 0.4\ mm^3$

2. To convert number of cells counted in 0.4 mm^3 into number of cells per ml, one needs a magnification factor (f_1) which represents:
   $f_1 = 1000\ mm^3/V = 1000\ mm^3/0.4\ mm^3 = 2500.0$

3. Since the sample was concentrated from 2 to 4 liters of seawater and then preserved in 3 to 10 ml of subsample, one also needs a concentration factor (f_2).
   f_2 = number of ml of seawater used for concentration / Number of ml of subsample preserved

4. Thus to convert the subsample phytoplankton counts to number of cells per ml of original seawater sample, it was necessary to multiply the count obtained from the mean (X) of the triplicate counts by f_1/f_2.
   $N = X \cdot \frac{f_1}{f_2}$

   Where N is the result in number of cells per milliliter.

Analyses of Inorganic Nutrients and Salinity

The inorganic nutrient analytic methods used in this research were based primarily on those described in "Seawater Analysis Methods" (Wallen, 1968).

The methods for nitrite, nitrate, phosphate and silicate analyses are outlined below.

All modern methods for determination of inorganic nitrite-nitrogen depend upon the diazotization of an amino compound followed by a coupling reaction to give an azo dye. The procedure for Bendschneider and Robinson (1952) is probably the most sensitive and trouble-free method for this determination. The nitrite in the seawater was allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with N-(1-naphthyl)-ethylenediamine to form a highly colored azo dye. The extinction of this dye was measured at 5400 Å in 10 cm cell, slit width 0.025 mm using a Beckman DU spectrophotometer.

Precise determination of inorganic nitrate-nitrogen in seawater is difficult. A method in which nitrate is quantitatively reduced to nitrite is advantageous, because nitrite can be easily determined by the sensitive and accurate diazotization method described above. The procedure of Morris and Riley (1963) with some modifications suggested by Wood et al. (1967) is one of the best methods of this kind. The nitrate in seawater was reduced quantitatively (over 90 percent) to nitrite when a 50 ml sample was run

through a reduction column containing amalgmated cadmium filings. Then the nitrite thus produced was determined by the same procedure as for nitrite analysis.

Most methods for measuring inorganic phosphate-phosphorus in seawater depend on the formation of a phosphomolybdate complex under strongly acid conditions. This complex is reduced to molybdenum blue and determined using spectrophotometry. The method of Murphy and Riley (1962) was applied to this analysis. A single solution (mixture of ascorbic acid, distilled water, EDTA, formic acid, sulfuric acid, potassium antimony tartrate, ammonium molybdate) was added to 50 ml of seawater sample. Under these acidic conditions phosphomolybdate was formed and reduced to molybdenum blue. The extinction of this blue solution was measured in a 10 cm cell at 8850 Å with a slit width of 0.025 mm.

The method of Grasshof (1964) was used for the determination with little modification. This method is based on the reaction with molybdate to form yellow silicomolybdic acid. The extinction of the yellow color is measured spectrophotometrically. A 50 ml seawater sample was first treated with a monochloroacetic acid solution followed by a sodium molybdate solution. After 3-4 hours incubation at room temperature, the yellow silicomolybdic acid solution was formed. Its extinction was measured at a wavelength of

3900 Å with a slit width of 0.030 mm.

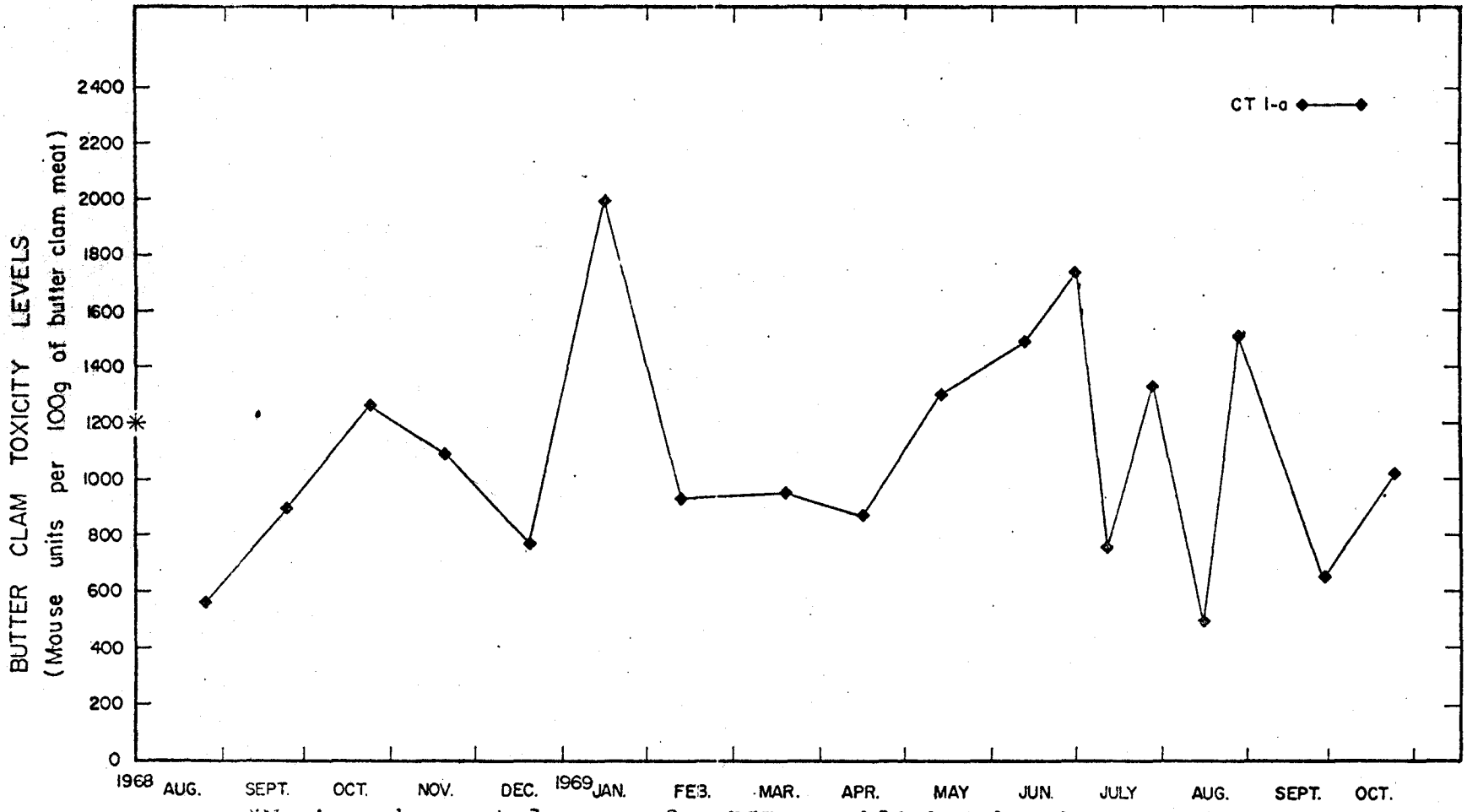
Salinity was measured with an inductive salinometer (Bissett-Berman Corporation Model 6220 Portable Laboratory Salinometer). Results are expressed in parts per thousand (‰). Inorganic nutrient concentrations are expressed in μg-at l^{-1}.

RESULTS

Seasonal Fluctuations of Butter-Clam Toxicity Levels

Seasonal variations of butter-clam toxicity levels did not follow any regular pattern; even variations at the three high-toxicity stations were not similar to each other (Figure 3, 4, 5 and 6).

Butter clams occasionally accumulated significant amounts (i.e. higher than the maximum human tolerance for PSP--1200 MU) of toxin during any season of the year, and occasionally rapidly lost and regained toxicity between two samplings. In general, all three stations showed comparatively low toxicity during August, September and December of 1968; and February, April and October of 1969, i.e. the toxicity levels in those months were lower than the mean of the three high-toxicity stations combined (1124 MU, Table 2). Station CT 1-b had the highest toxicity levels with a mean of 1521 MU, and was peculiar in that toxicities appeared in alternate months (Figure 4). From August, 1968 to May, 1969 station CT 1-a (Figure 3) had fluctuations similar to those of CT 1-b, but periods of uptake of the toxin did not agree well with each other from June to October, 1969. Furthermore, toxicity levels at CT 1-a were usually lower than CT 1-b. Station CT 2 had the lowest levels in this group (Figure 5); toxicity levels



*Maximum human tolerance for PSP established by the U.S. Food and Drug Administration.

Figure 3. Butter-clam toxicity levels at station CT 1-a.

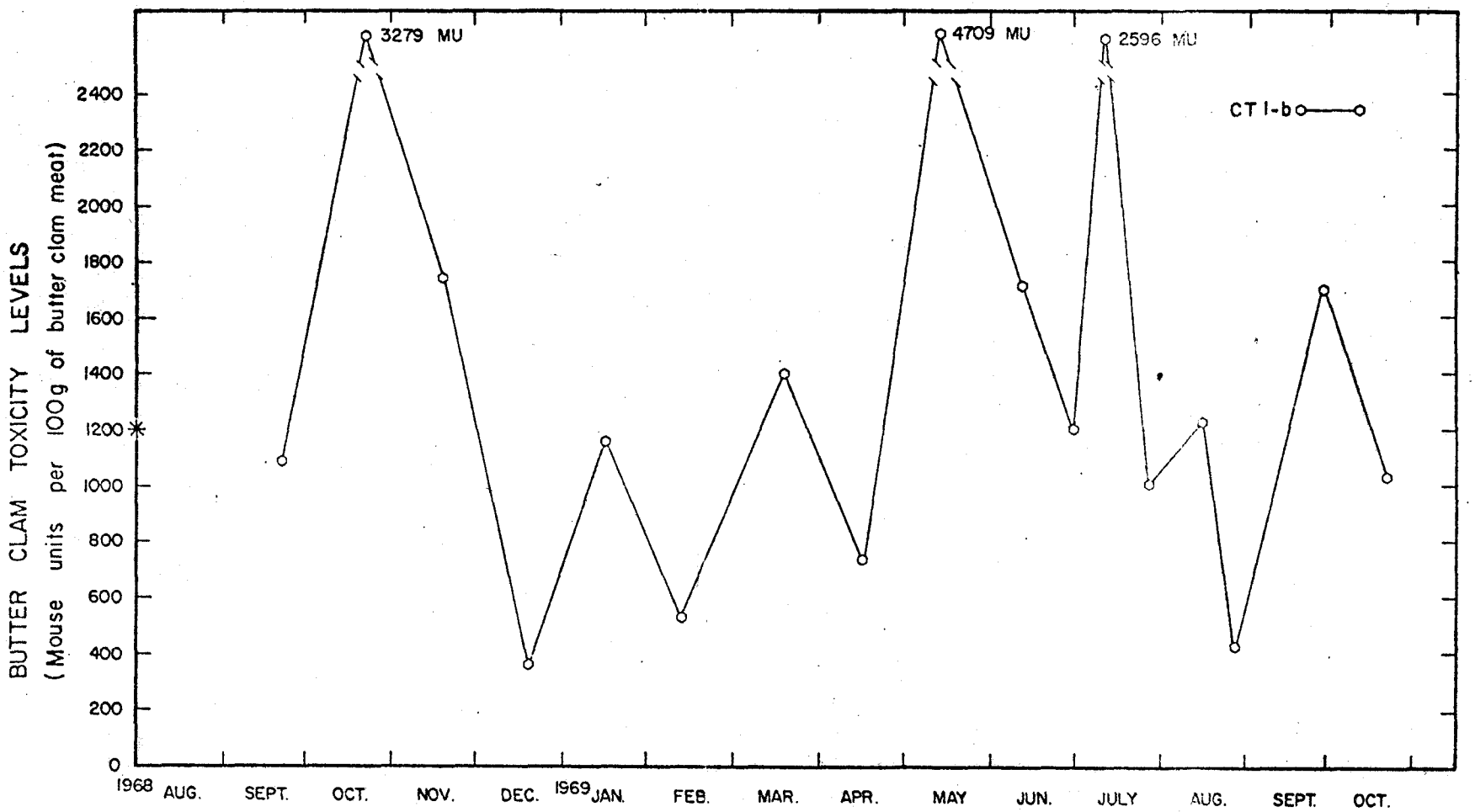


Figure 4. Butter-clam toxicity levels at station CT 1-b.

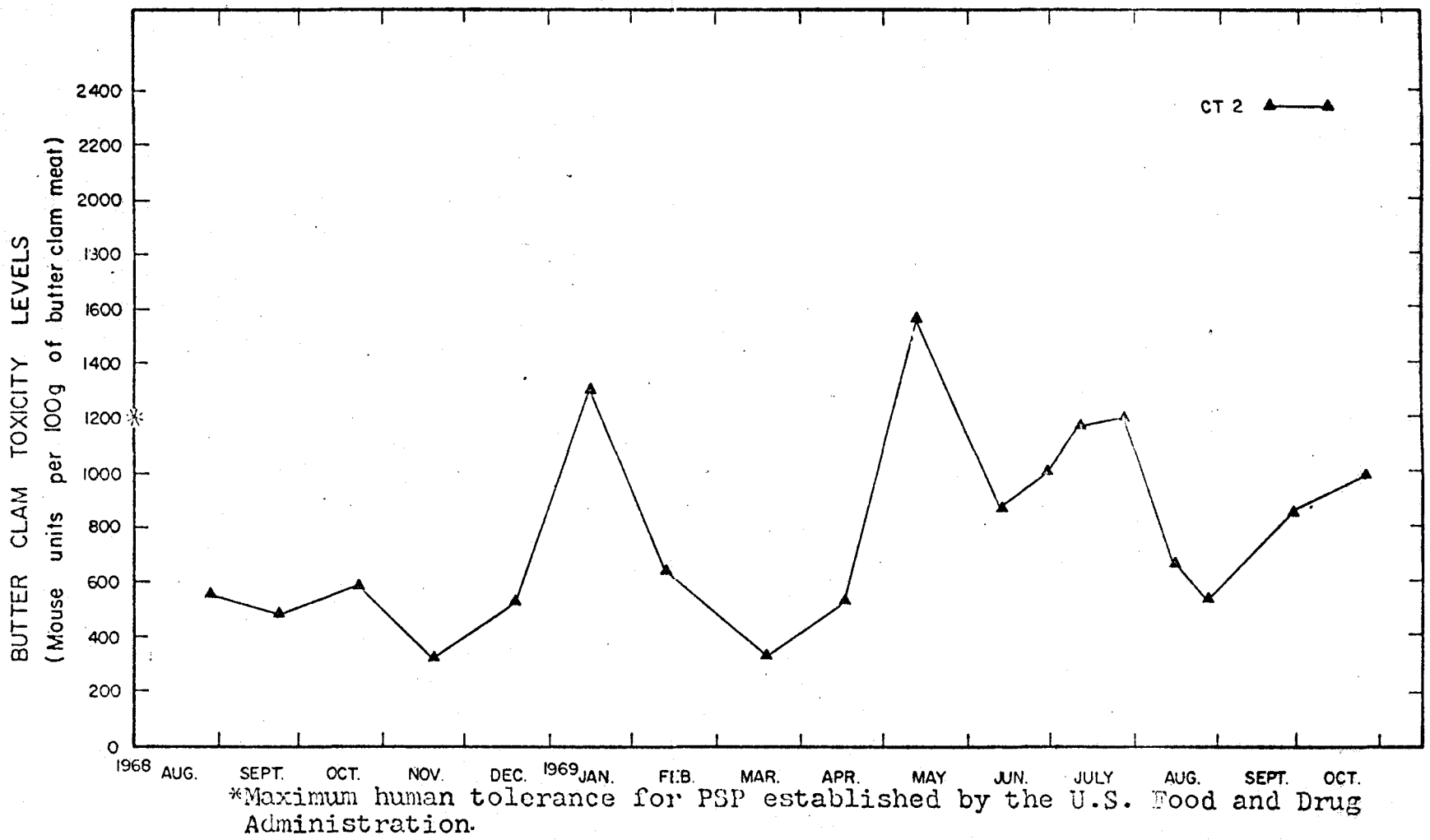


Figure 5. Butter-clam toxicity levels at station CT 2.

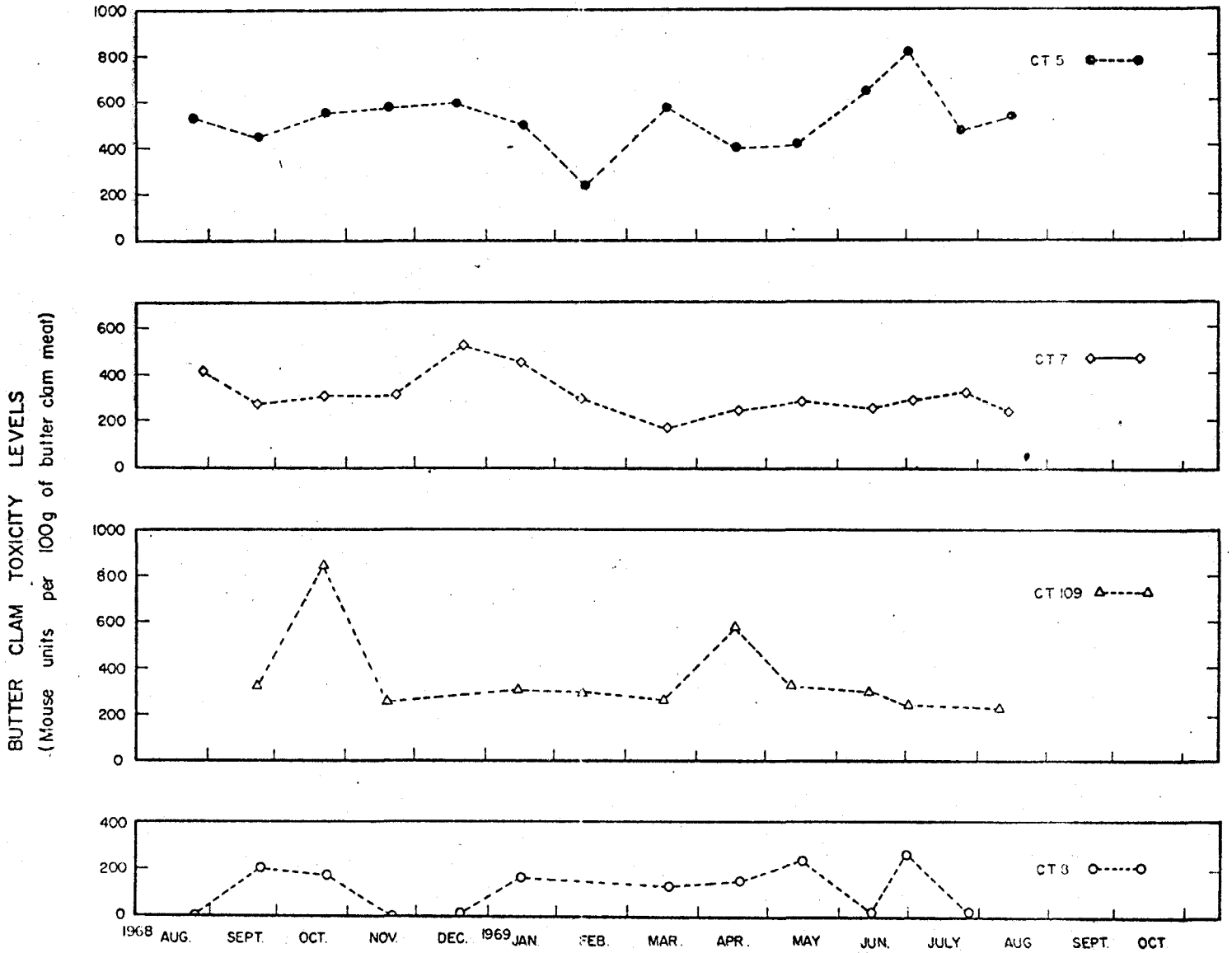


Figure 6. Butter-clam toxicity levels at moderate-toxicity and low-toxicity stations.

Table 2. Butter-clam toxicity levels at three high-toxicity stations.

Sampling Date	CT 1-a Toxicity (MU)	CT 1-b Toxicity (MU)	CT 2 Toxicity (MU)
1968 Aug. 24	556.0		556.0
Sept. 22	892.1	1089.2	491.0
Oct. 23	1260.0	3279.0	590.8
Nov. 19	1086.0	1740.0	307.0
Dec. 19	759.0	361.0	530.0
1969 Jan. 16	1987.0	1167.0	1319.0
Feb. 13	926.0	512.0	643.0
Mar. 19	947.0	1402.0	325.0
Apr. 16	873.0	730.0	525.0
May 13	1295.0	4709.4	1582.0
June 12	1487.0	1710.0	876.0
June 30	1724.0	1200.0	1012.0
July 11	754.0	2596.0	1171.0
July 27	1334.0	999.0	1200.0
Aug. 15	493.0	1221.0	657.0
Aug. 27	1508.0	418.0	532.0
Sept. 27	651.0	1695.0	854.0
Oct. 23	1014.0	1030.0	995.0
Sample size	18	17	18
Mean	1085.9	1521.1	787.0
Mid-range	493.0-1987.0	361.0-4709.4	307.0-1582.0
Std. dev.	410.5	1111.4	359.7
Std. error	96.8	269.6	84.8
Confidence limits for the mean at 95 percent level	± 204.1	± 571.5	± 178.9

Three high-toxicity stations CT 1-a, CT 1-b and CT 2, combined

Mean	1124.0	Std. Error	103.6
Std. dev.	754.2	Confidence limits for the mean at 95 percent level	± 208.0

Table 3. Butter-Clam toxicity levels at a moderate-toxicity station.

Sampling Date	CT 5 Toxicity (MU)
1968 Aug. 25	531.0
Sept. 22	438.4
Oct. 22	540.0
Nov. 19	575.0
Dec. 18	592.0
1969 Jan. 17	488.0
Feb. 13	230.0
Mar. 18	563.0
Apr. 17	394.0
May 14	407.0
June 13	629.0
July 2	796.0
July 25	456.0
Aug. 14	517.0
Sample Size	14
Mean	511.2
Mid-range	230.0-796.0
Std. dev.	130.7
Std. error	34.9
Confidence limits for the mean at 95 percent level	± 75.4

Table 4. Butter-Clam toxicity levels at three low-toxicity stations.

Sampling Date	CT 7 Toxicity (MU)	CT 109 Toxicity (MU)	CT 8 Toxicity (MU)
1968 Aug. 28	400.0		0.0
Sept. 21	258.6	321.5	207.5
Oct. 22	302.6	848.0	173.0
Nov. 20	298.0	251.0	0.0
Dec. 17	508.0		0.0
1969 Jan. 15	443.0	296.0	163.0
Feb. 11	286.0		
Mar. 17	159.0	258.0	131.0
Apr. 18	239.0	570.0	139.0
May 14	279.0	295.4	232.0
June 14	243.0	276.0	0.0
July 1	267.0	229.0	266.0
July 26	306.0		0.0
Aug. 13	224.0	211.0	0.0
Sample size	14	10	13
Mean	300.9	355.6	100.9
Mid-range	159.0-508.0	211.0-848.0	0.0-266.0
Std. dev.	91.9	200.1	103.4
Std. error	24.6	63.3	28.7
Confidence limits for the mean at 95 percent level	± 53.1	± 143.2	± 62.5

Three low-toxicity stations CT 7, CT 109 and CT 8 combined

Mean	245.4	Std. error	27.9
Std. dev.	169.5	Confidence limits for the mean at 95 percent level	± 56.6

showed variations but less than CT 1-a and CT 1-b.

CT 5, a station with moderate toxicity, showed little change in toxicity levels, but demonstrated a relatively low level of toxicity in February 1969 (230 MU) and a relatively high level in July 1969 (796 MU) (Figure 6), as compared to the mean value for this station (511 MU, Table 3).

CT 8 had the lowest toxicity levels throughout the investigation (Figure 6). Toxicity was not detectable most of the time, and was usually not higher than the mean of the three low-toxicity stations (245 MU, Table 4), with the exception of July 1969 (266 MU). Station CT 109 and CT 7 usually did not show any appreciable toxicity (Figure 6), except that in December 1968 (508 MU) at CT 7, and in October 1968 (848 MU) and April 1969 (570 MU) at CT 109, the toxicity levels were much higher than the mean of the three low-toxicity stations (Table 4).

Seasonal Fluctuations of Phytoplankton Populations

An attempt was made to correlate phytoplankton populations and hydrographic conditions at various depths in the water column (within the euphotic zone) with toxicity in the adjacent clam beds. Because of mixing processes of turbulence, waves and tides and the vertical migration of phytoplankton populations, these parameters at various depth may have opportunities to affect intertidal clams.

Thus, in the examination of the relationship between clam toxicity levels and these parameters, it seems more reasonable to use the mean of the data obtained for all four depths than to use the results from each depth. For comparison with the results of the mean the surface samples were also used for analysis; two graphs were plotted for each parameter, one for the surface and another for the mean.

The patterns of fluctuations within the major groups of phytoplankton were basically similar (see Appendix B). To describe the changes for each genus separately was time consuming and seemed superfluous. Instead, all genera were grouped into two categories, diatoms and dinoflagellates, and the changes of these two groups are described (see Appendix B). Diatoms were the predominant group in the plankton populations examined. The monthly results of the major genera are also listed in Appendix B.

Two patterns of diatom fluctuation are observed in Figure 7 and 8. The changes at the three high-toxicity stations are similar (solid lines). From August, 1968 to February, 1969 diatom populations were extremely low (the counts of some samples were almost zero). The highest peak occurred in the late spring, but the peak of station CT 1-a was a little later than that at CT 1-b and CT 2. Station CT 5, which is of moderate toxicity, had a diatom fluctuation similar to the three high-toxicity stations.

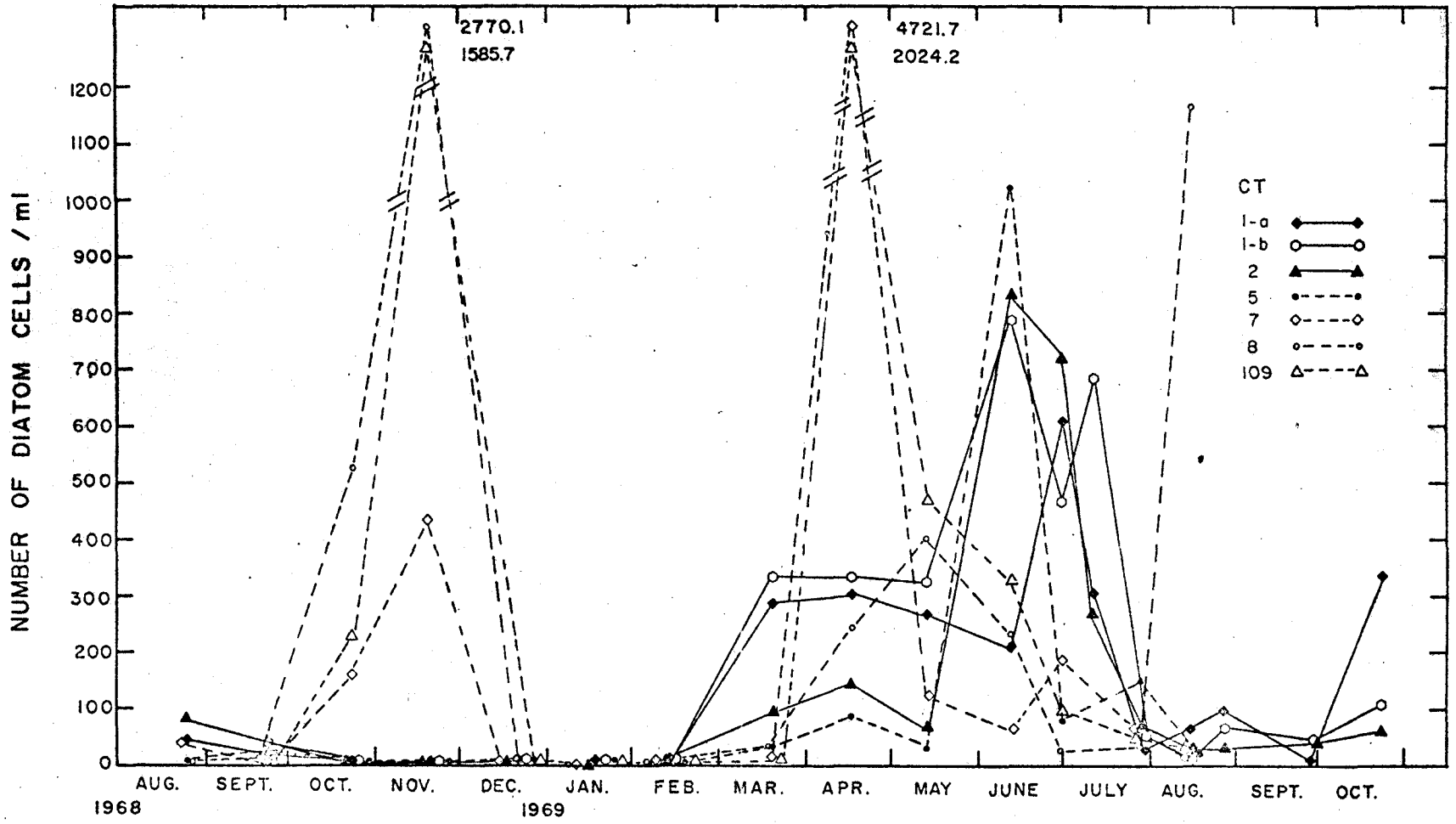


Figure 7. Fluctuations of diatom populations in surface samples.

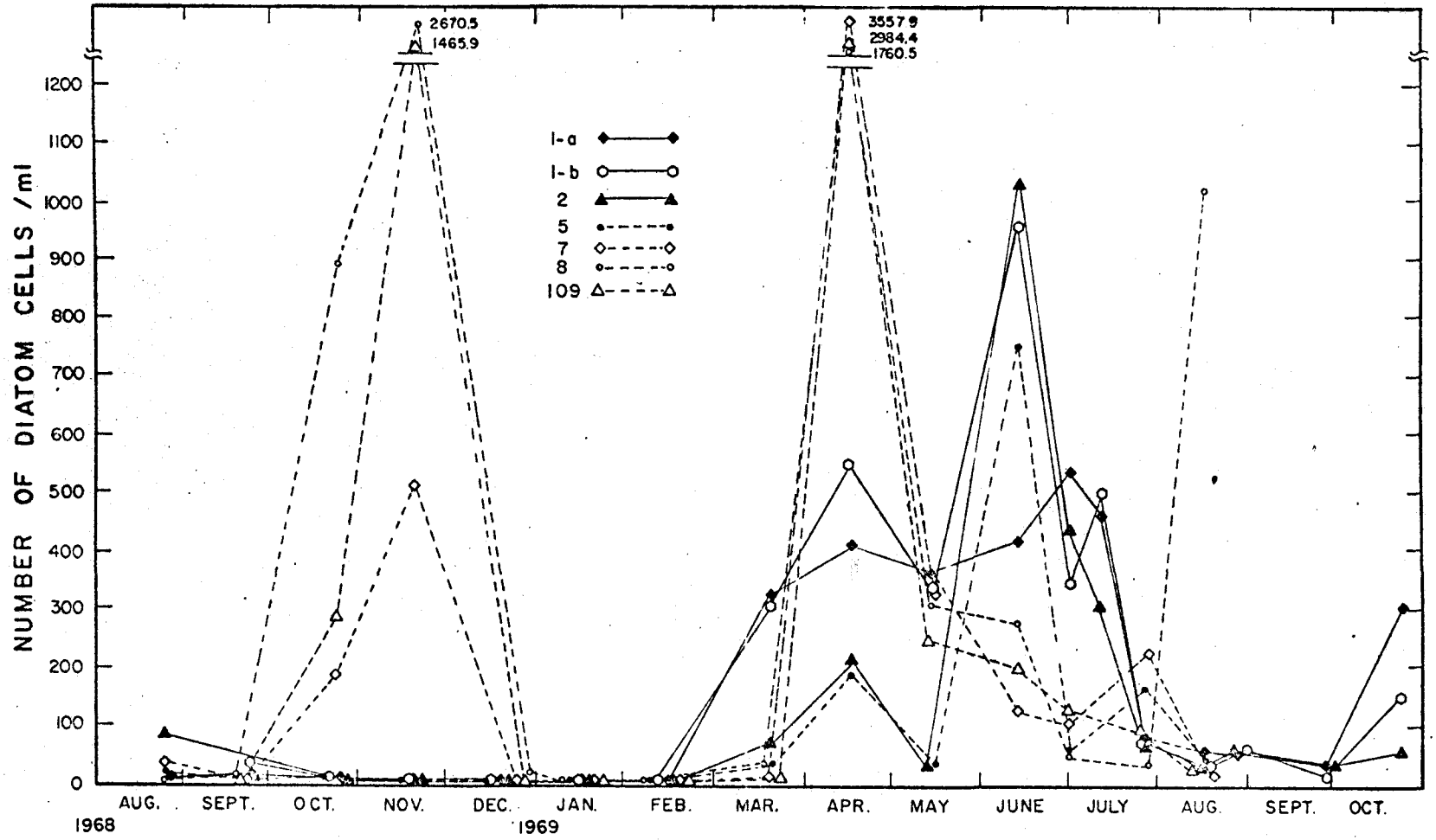


Figure 8. Mean fluctuations of diatom populations sampled from four depths.

The density of the dinoflagellate populations was much smaller than that of the diatoms. The fluctuations are shown in Figure 9 and 10. The maxima of dinoflagellates occurred in spring and summer; the organisms were extremely scarce at other times of the year. Moreover, the peak of dinoflagellates did not occur at the same time as the diatom peak, and the former tended to occur a little later. At station CT 7, CT 109 and CT 8 there was, in addition, a fall bloom of diatoms which was not followed by a dinoflagellate flowering.

A dinoflagellate species tentatively identified as Gonyaulax sp., was found in the phytoplankton samples, but it only represented a small proportion of the dinoflagellates in the sample. For simplification purposes, it will be called Gonyaulax sp. in this dissertation (see Discussion, Page 63). Counts for Gonyaulax sp. are listed in Appendix B. This is of special interest here because Gonyaulax sp. is the causative organism of California mussel poisoning (Sommer et al., 1937).

The mean for diatom and dinoflagellate numbers at the four sampling depths was similar to the value for the surface sample.

Hydrographic Conditions in the Research Areas

In general, the annual pattern of changes of salinity, inorganic nutrients and water temperature was similar at

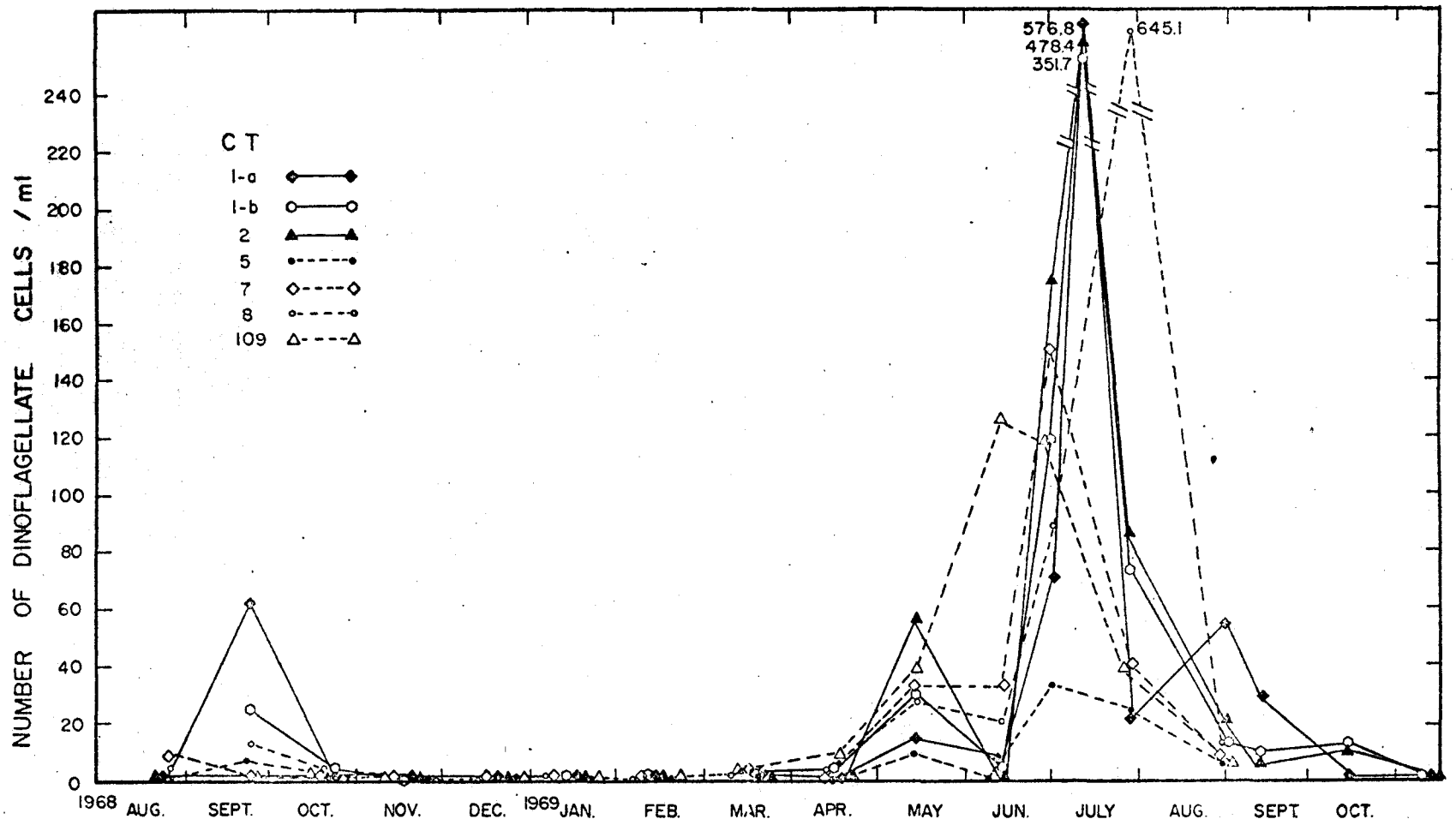


Figure 9. Fluctuations of dinoflagellate populations in surface samples.

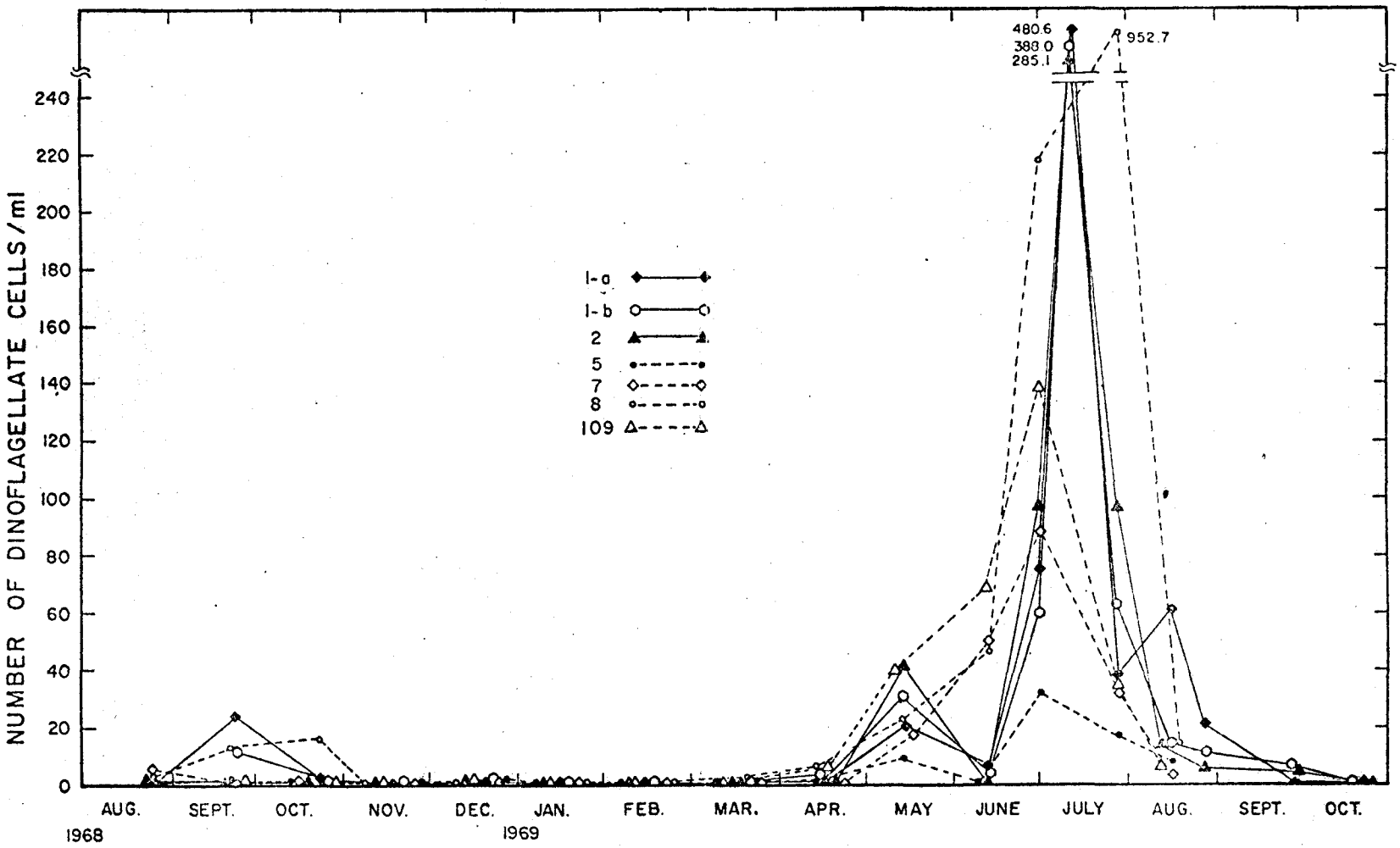


Figure 10. Mean fluctuations of dinoflagellate populations sampled from four depths.

all stations. Salinities, nitrates, phosphates and silicates showed relatively high values with small variations during the winter, and relatively low values with large variations during the summer. The decrease of salinity in the summer was caused mainly by fresh water run-off from streams, rain, and melting glaciers. By the middle of summer the lowest salinity was reached. Salinity began to increase again at the start of winter. The range of variations is shown in Figure 11 and 12. Nitrate was generally abundant, but its range varied from almost undetectable to 15.5 μg-at l^{-1} (Figure 13 and 14). Phosphate concentration was never as high as nitrate; it had a range between 0.16 and 1.50 μg-at l^{-1} (Figure 15 and 16). Silicate was another abundant inorganic nutrient; the concentrations were between 6.55 and 42.91 μg-at l^{-1} during the summer and rose sharply to 70.00 μg-at l^{-1} in the winter (Figure 17 and 18.

The changes of nitrite followed another pattern (Figure 19 and 20). Nitrite increased after the period of the most rapid utilization of nitrate by phytoplankton populations in the fall, and then decreased and was minimal at approximately the period of maximum nitrate. The nitrite concentrations were low at all times, and the fluctuations were not as marked as those of nitrate. Concentrations ranged from 0.049 to 0.546 μg-at l^{-1}.

The seasonal water temperatures followed a consistent pattern (Figure 21 and 22). Minimum temperatures were 0.8°C to 3.3°C from January to March and slowly increased to maximum temperatures of 7.5°C to 18°C from June through September. Thermal stratification was occasionally observed during the summer months.

The graph of the mean of any hydrographic parameter at the four depths sampled is similar to the graph of that parameter at the surface. The means and standard deviations of phytoplankton populations and hydrographic parameters are shown in the Tables of correlation coefficients.

Butter-Clam Toxicity Levels, Phytoplankton Populations, and Hydrographic Conditions.

Fluctuations of the more abundant phytoplankton genera (see Appendix B) and hydrographic parameters were compared with fluctuations of butter-clam toxicity levels. Neither phytoplankton populations nor hydrographic parameters showed any clear relationship to toxicity levels. On the other hand the curves in Figure 7, 8, 9 and 10 showing variations in phytoplankton populations show an inverse relationship with the curves for most hydrographic parameters (Figure 11 to Figure 22). For example, an obvious negative correlation was found between the phytoplankton numbers and inorganic nutrient concentrations.

A correlation analysis was also used to examine the

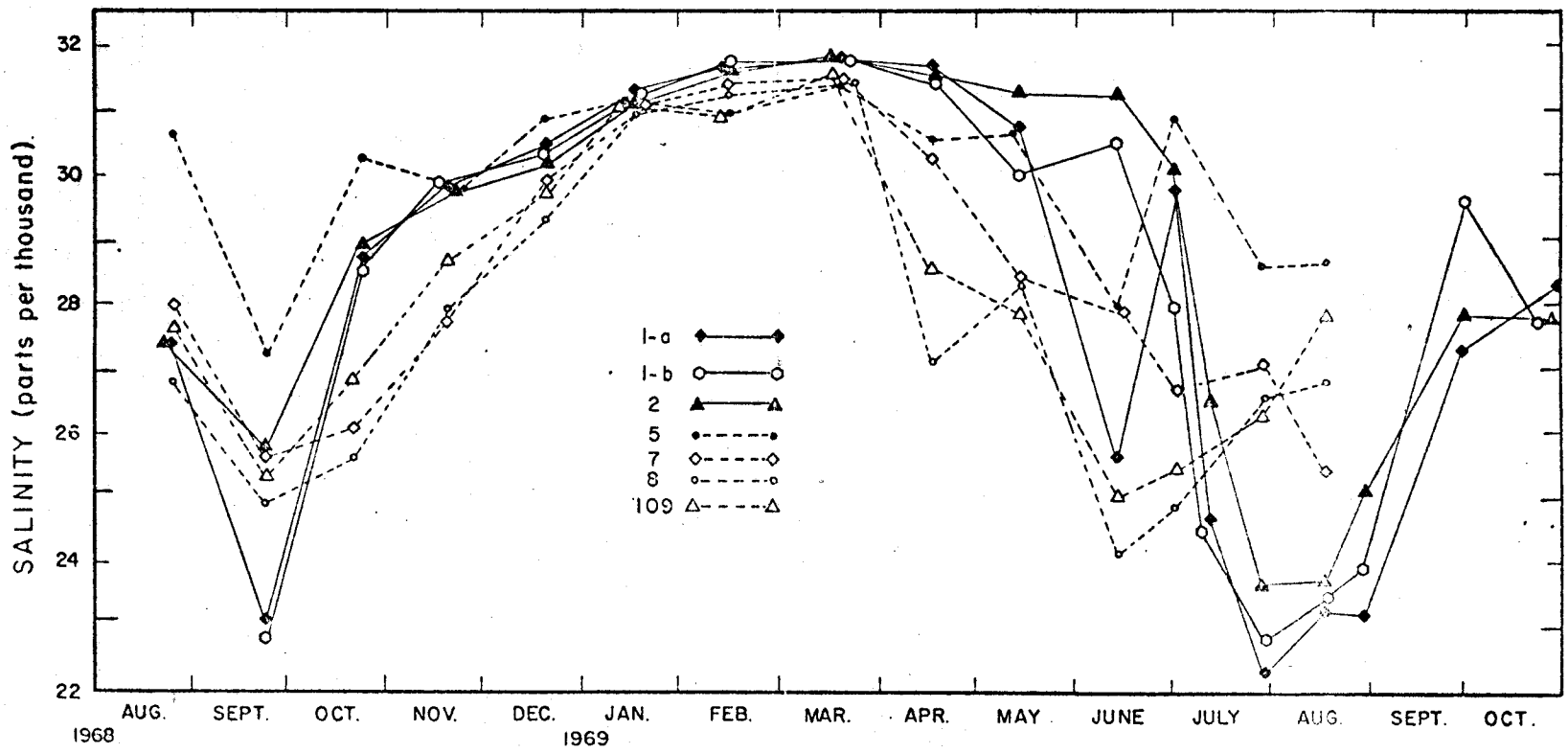


Figure 11. Salinity levels of surface samples.

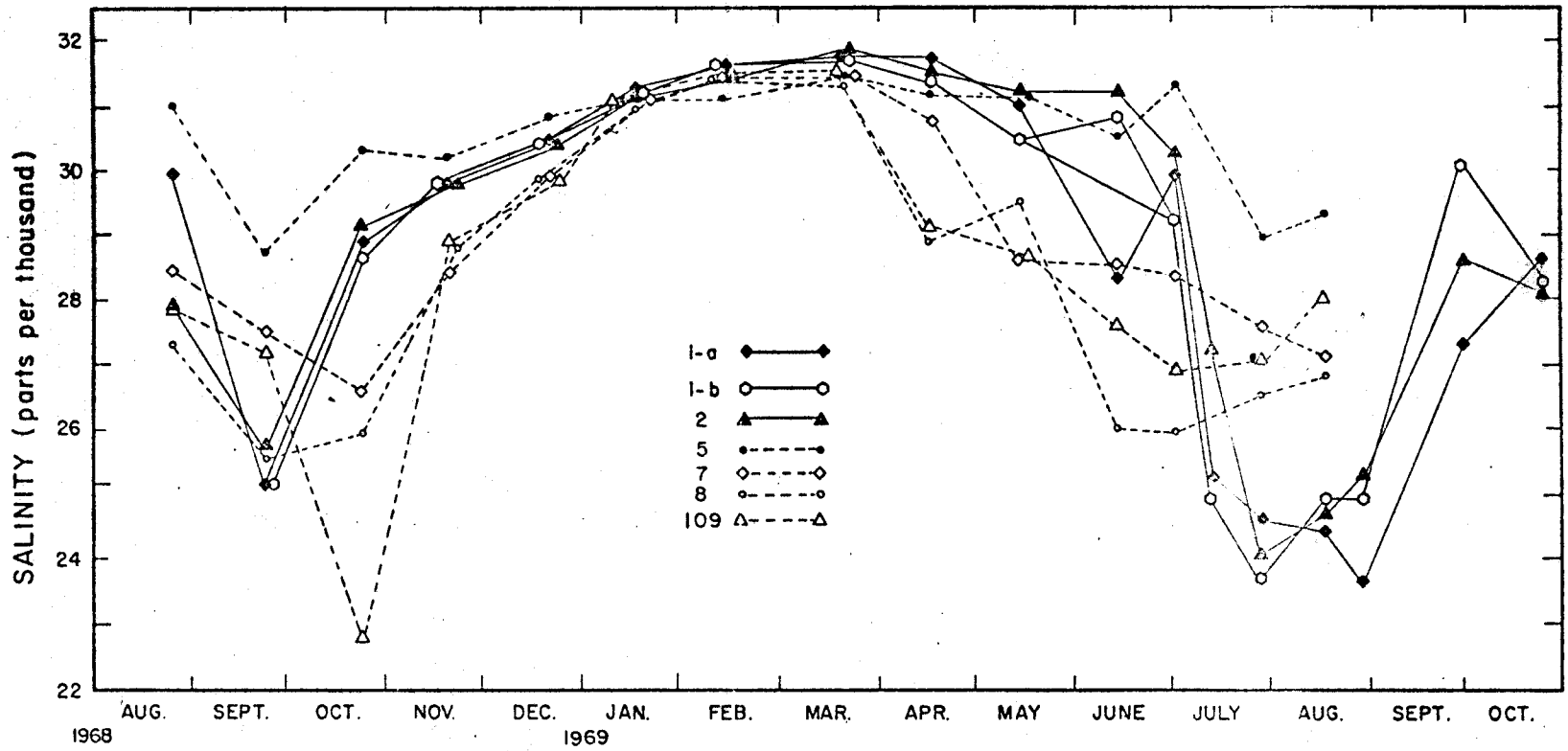


Figure 12. Mean salinity levels of samples from four depths.

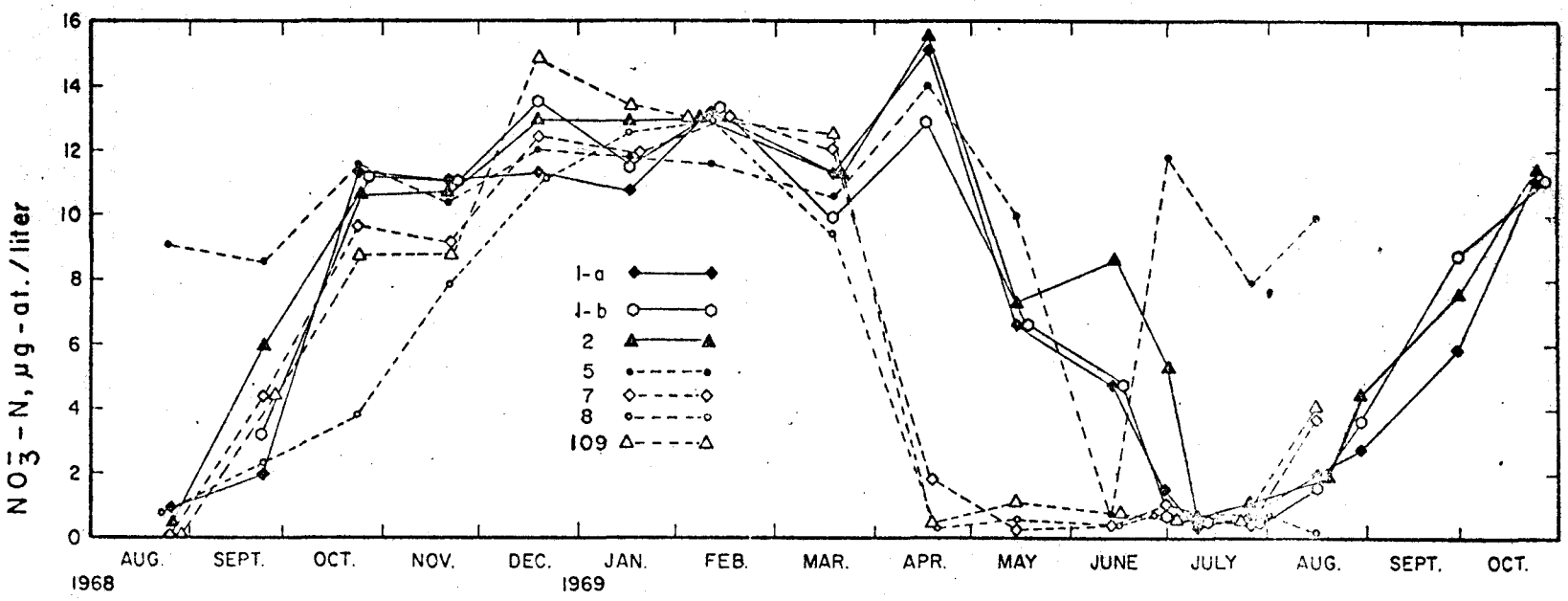


Figure 13. Concentrations of inorganic nitrate-nitrogen in surface samples.

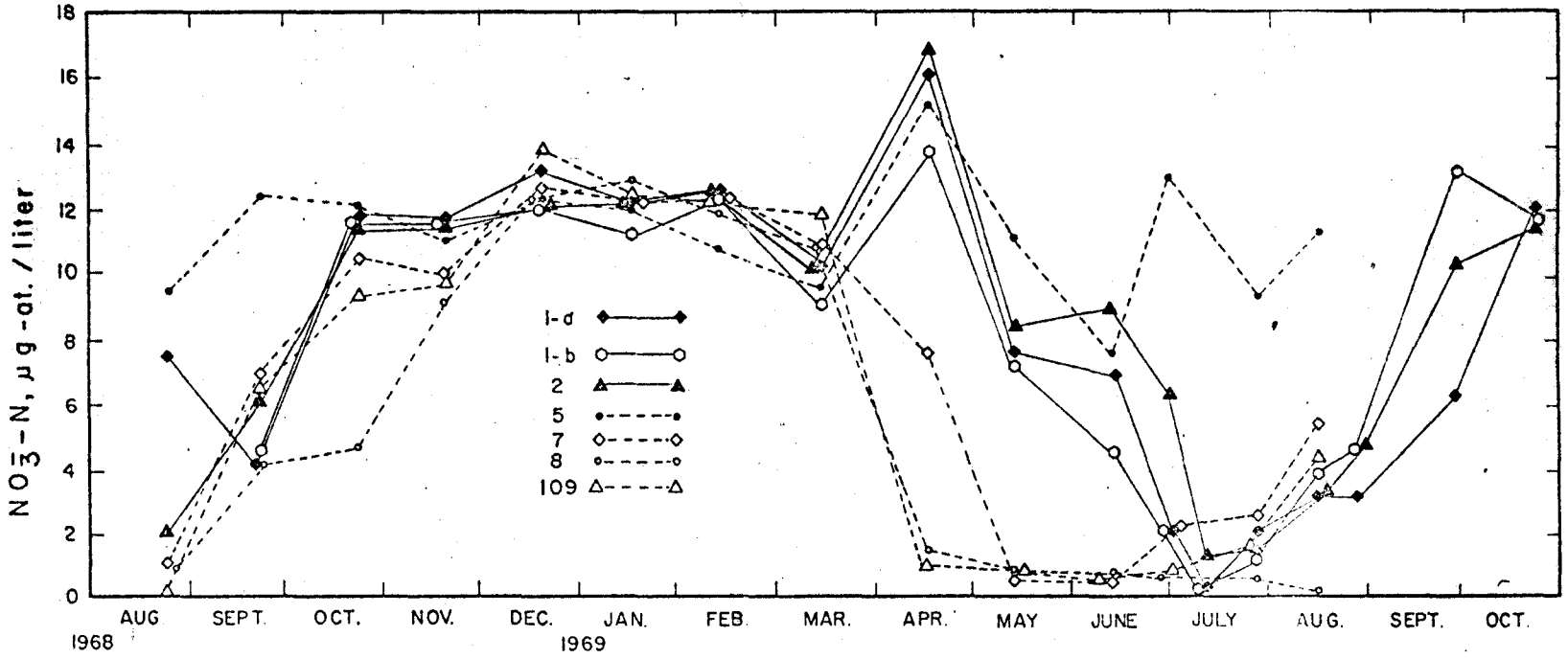


Figure 14. Mean concentrations of inorganic nitrate-nitrogen in samples from four depths.

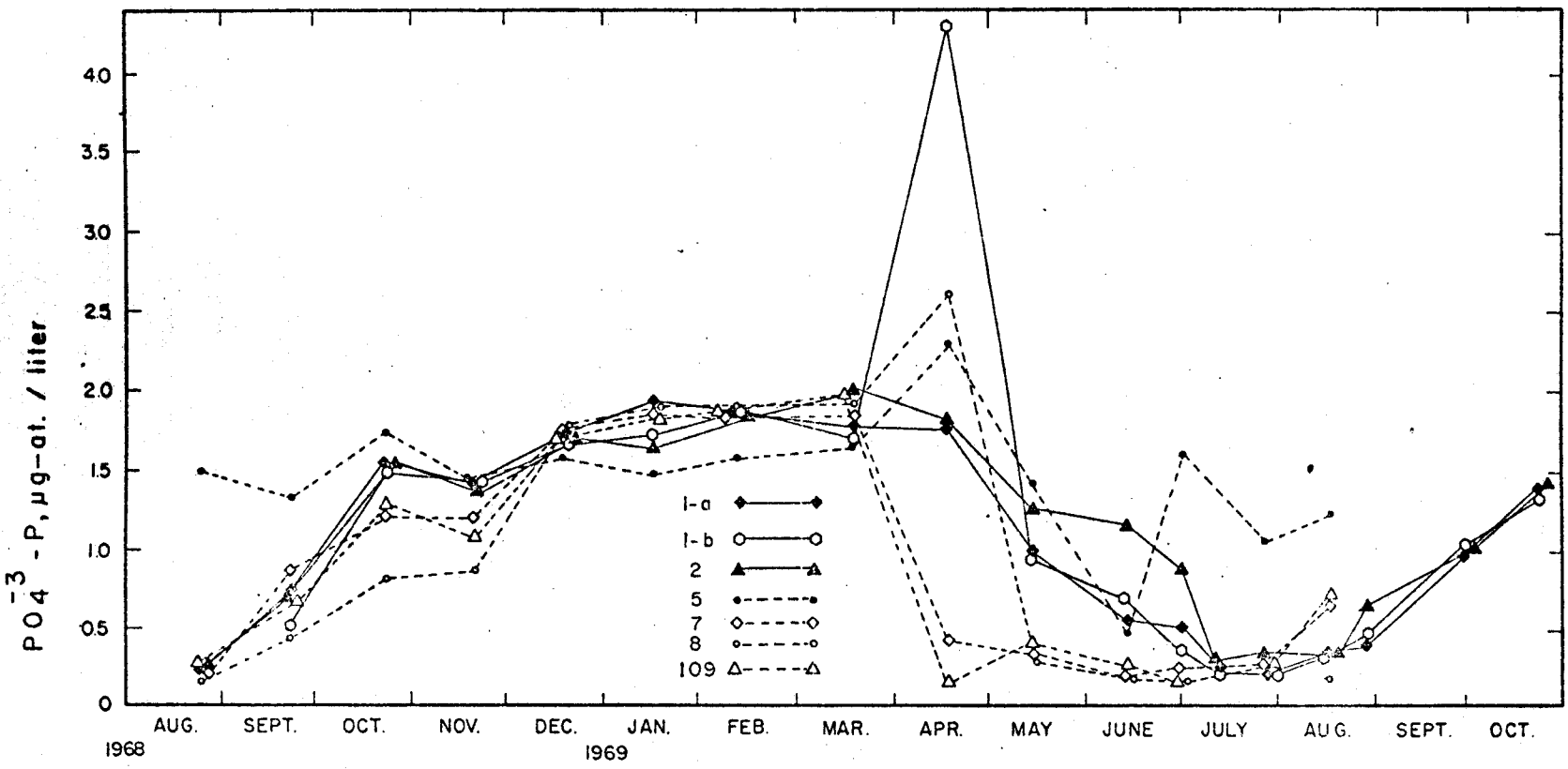


Figure 15. Concentrations of inorganic phosphate-phosphorus in surface samples.

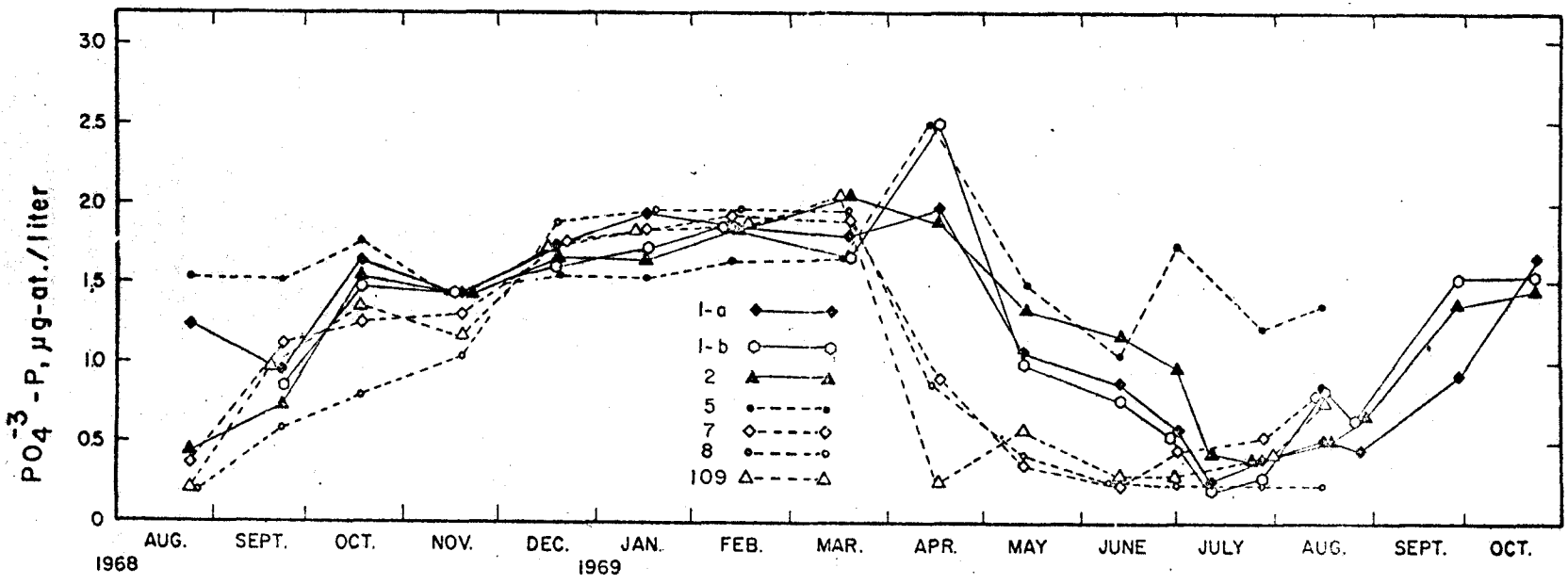


Figure 16. Mean concentrations of inorganic phosphate-phosphorus in samples from four depths.

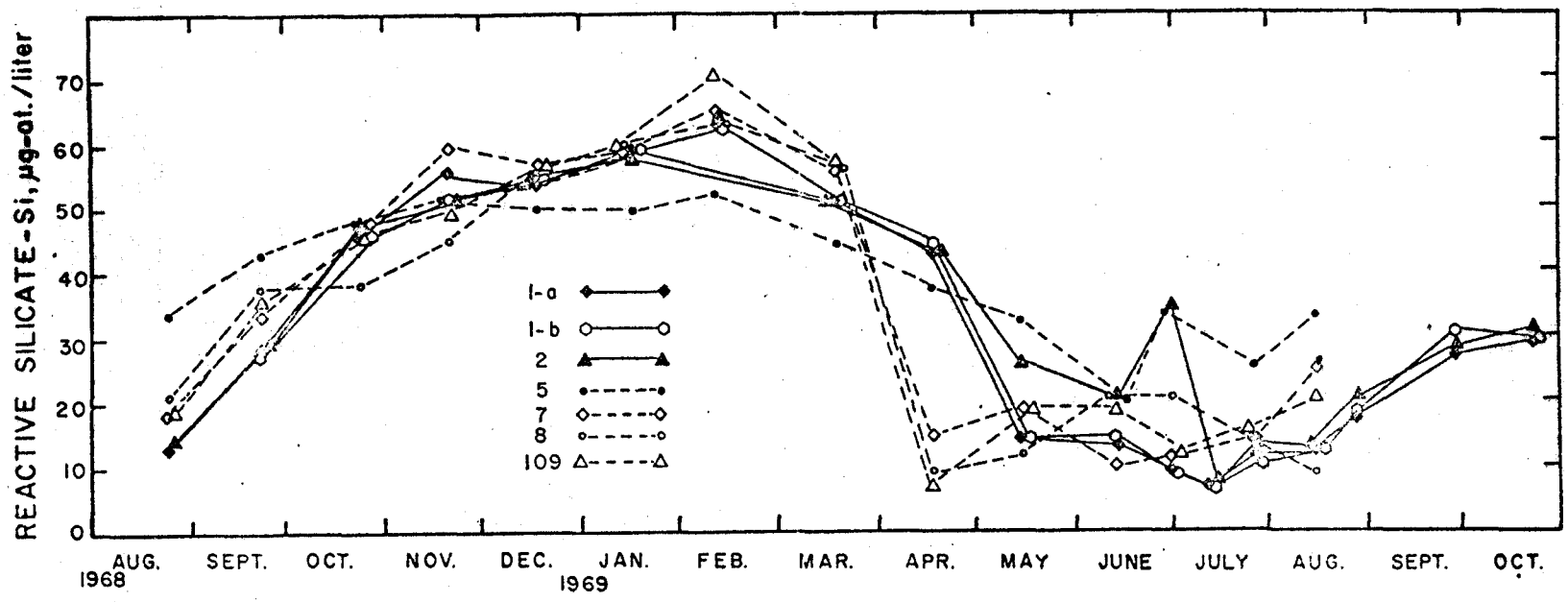


Figure 17. Concentrations of reactive silicate-silicon in surface samples.

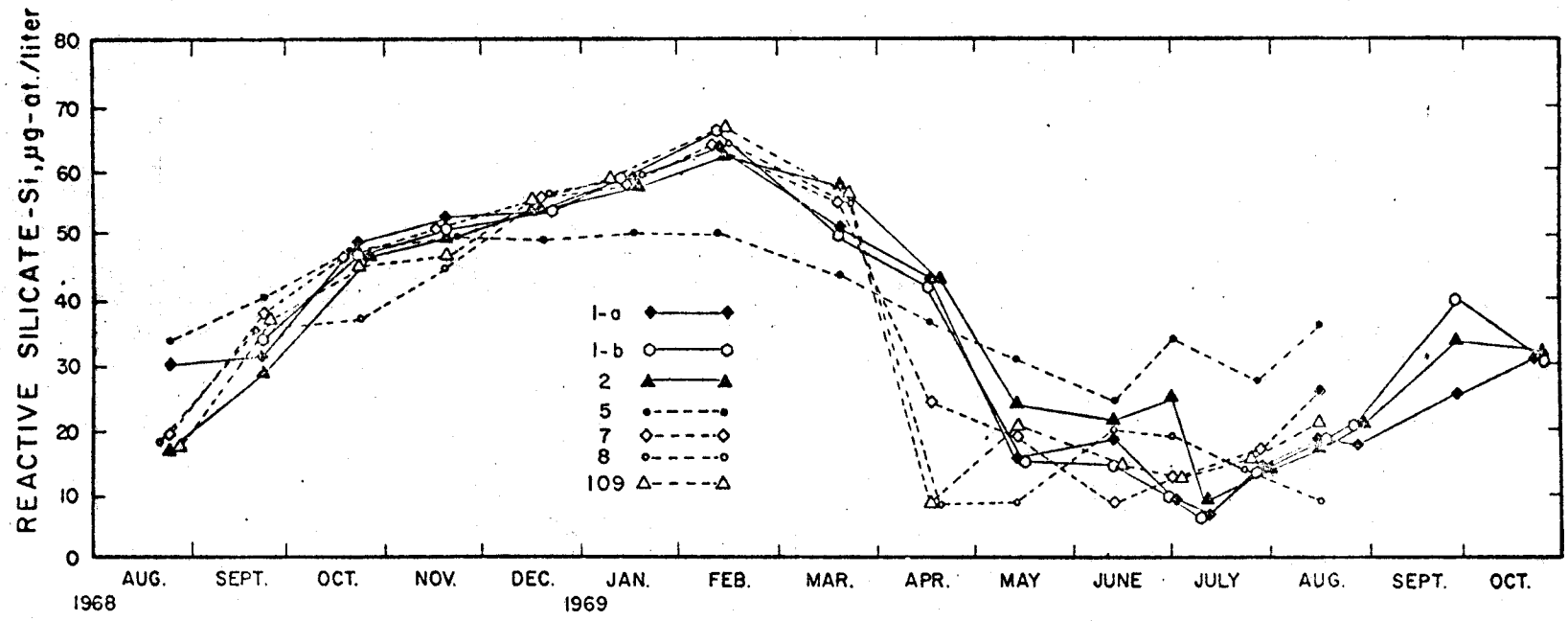


Figure 18. Mean concentrations of reactive silicate-silicon in samples from four depths.

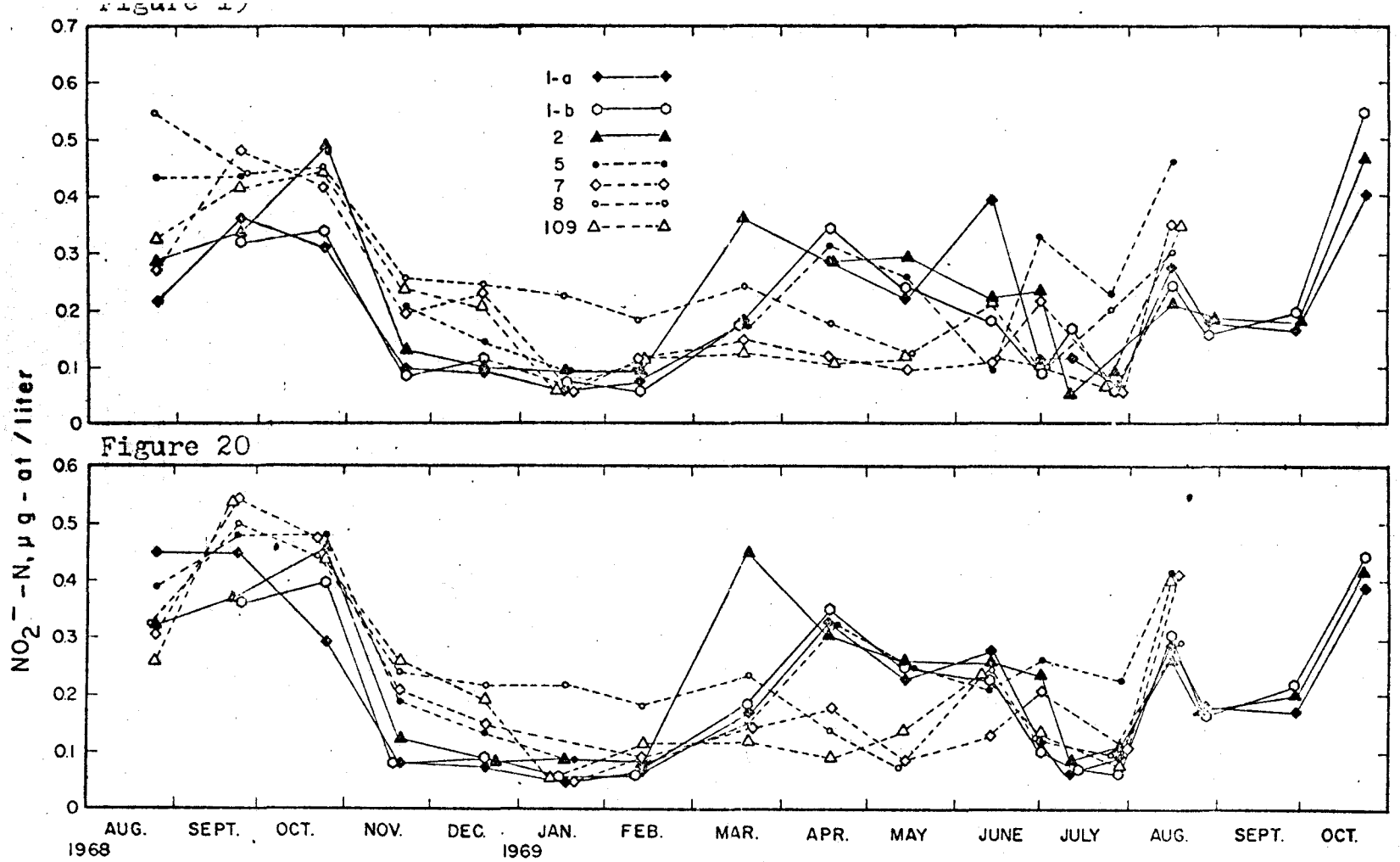


Figure 20

Figure 19. Concentrations of inorganic nitrite-nitrogen in surface samples.

Figure 20. Mean concentrations of inorganic nitrite-nitrogen in samples from four depths.

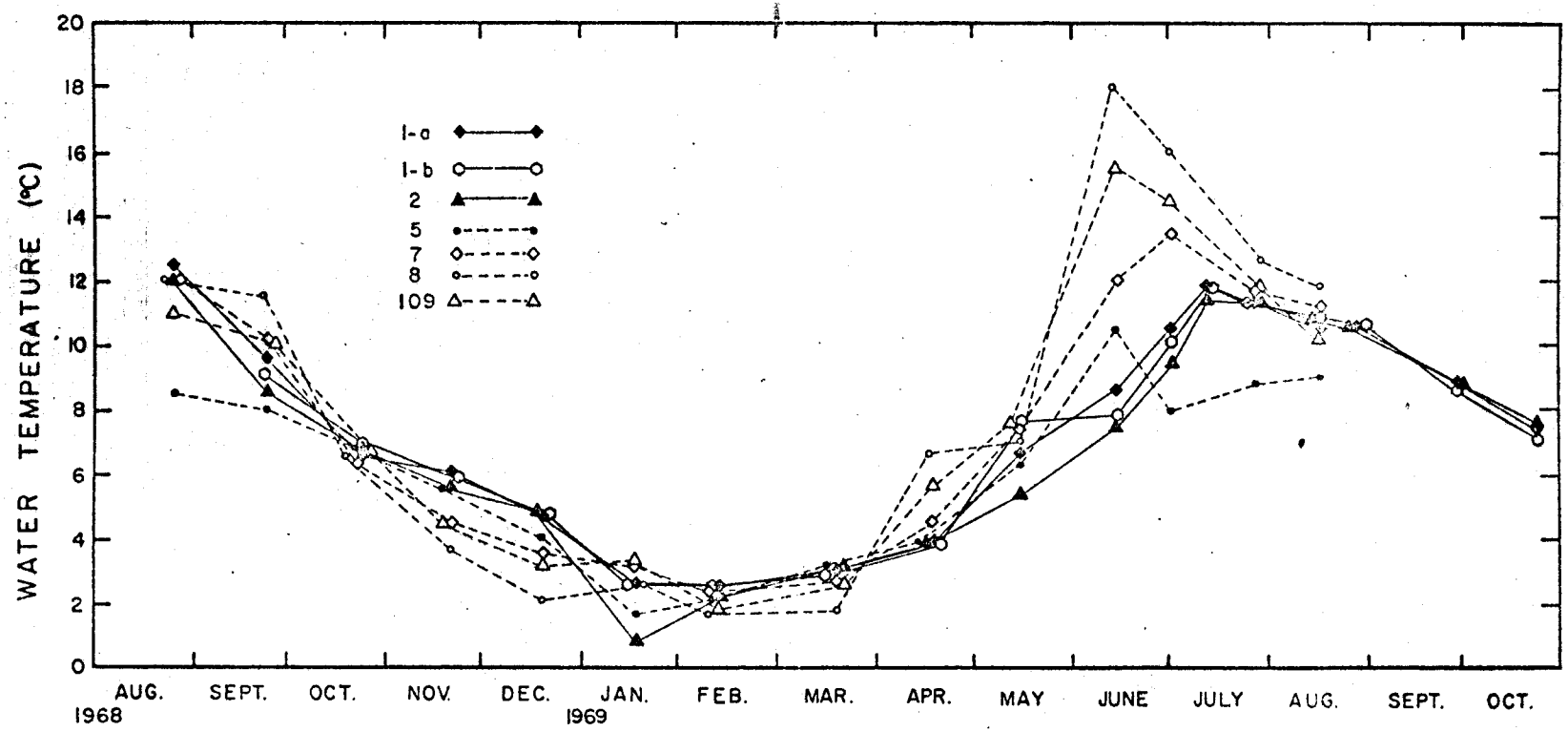


Figure 21. Fluctuations of surface water temperatures.

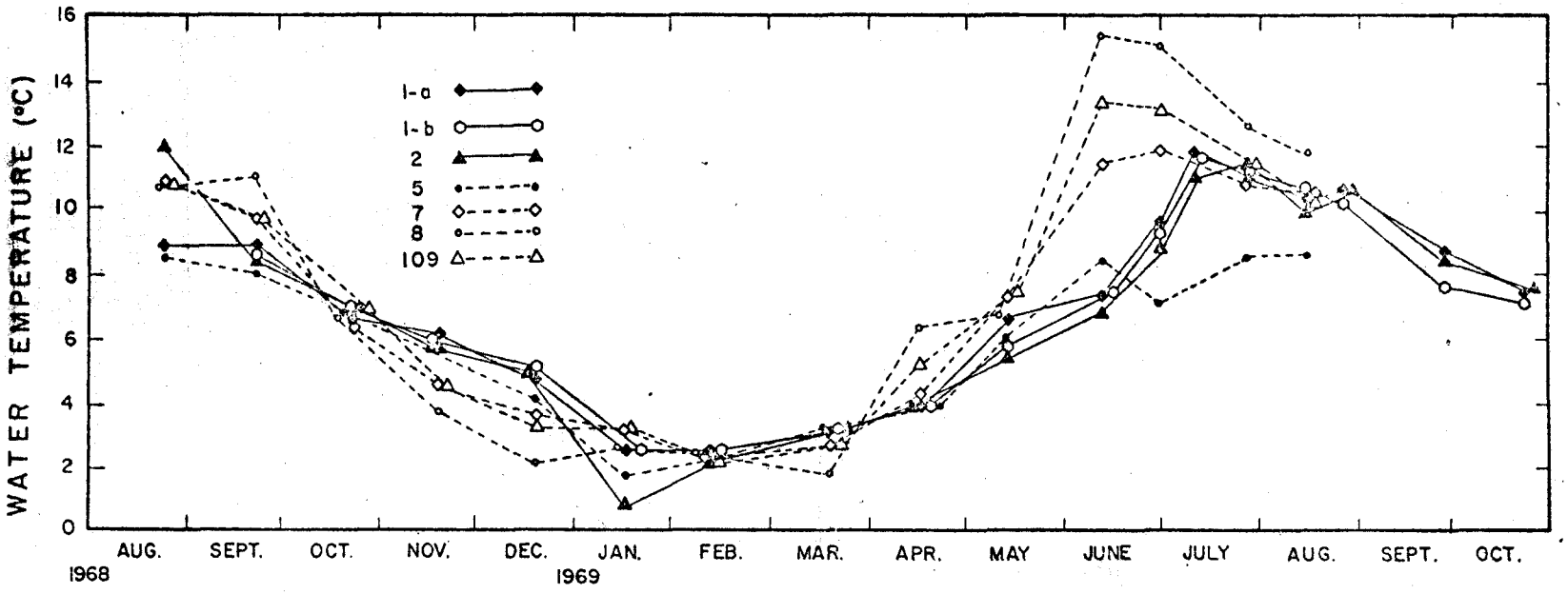


Figure 22. Mean fluctuations of water temperatures from four depths.

relationships between butter-clam toxicity levels, phytoplankton populations and hydrographic parameters. Neither phytoplankton populations nor hydrographic parameters showed a consistently significant correlation with the butter-clam toxicity levels (Tables 5, 6, 7 and 8).

A simple ocrrelation coefficient was calculated for the relationship between each independent variable (phytoplankton populations, hydrographic parameters) and the dependent variable (butter-clam toxicity levels). A partial correlation coefficient was also calculated between these variables. A multiple correlation coefficient (R) was calculated between each dependent variable and all of the independent variables. R was then tested by the F-value. The significance of these coefficients (simple, partial and multiple correlation coefficients) was tested at the 5 percent and 1 percent levels (Tables in p. 174 and p. 246, Snedecor, 1956). The results are tabulated in Tables 5, 6, 7 and 8.

Since the data for moderate and low-toxicity stations were incomplete (no observations made at those stations during the winter), the correlation analysis was made for two groups (the three most toxic stations, and all stations combined).

Although there was no consistent relationship between phytoplankton and clam toxicity, the phytoplankton showed significant correlation with hydrographic parameters. The results of correlation analysis are shown in Tables 9, 10,

Table 5. Correlation coefficients between butter-clam toxicity levels and phytoplankton populations for three high-toxicity stations combined.

A. Surface samples.

Dependent variable			Sample size		Independent variable: Diatoms	Dinoflagellates	Gonyaulax sp.	Total cell number	R	F-Test
				[1] $\bar{X}$	160.2	44.4	3.2	207.4	[5]	
				[2] S	224.3	113.6	6.8	282.7		
Clam toxicity	$\bar{X}$	1124.0	52	[3]	0.251	0.097	0.214	0.236	0.305	1.207
	S	754.2		[4]	0.074	0.057	0.176	-0.066		

B. Means of four depths.

Dependent variable			Sample size		Independent variable: Diatoms	Dinoflagellates	Gonyaulax sp.	Total cell number	R	F-Test
				$\bar{X}$	171.2	35.9	2.1	209.6		
				S	239.8	92.6	4.2	280.3		
Clam toxicity	$\bar{X}$	1124.0	52		0.205	0.145	0.191	0.221	0.269	0.917
	S	754.0			0.109	0.103	0.118	-0.109		

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient

Table 6. Correlation coefficients between butter-clam toxicity levels and hydrographic conditions for three high-toxicity stations combined.

A. Surface samples

Dependent variable		Sample size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-R Test
	[1] $\bar{X}$		28.220	7.372	0.204	1.123	32.280	7.37	
	[2] S		3.108	4.870	0.123	0.749	18.789	3.26	
Clam toxicity	$\bar{X}$ 1124.0	52	[3] 0.029	- 0.103	0.007	- 0.114	- 0.186	0.005	
	s 754.2		[4] 0.035	0	0	- 0.119	- 0.270	- 0.288*	[5] 0.403 1.455

B. Means of four depths

Dependent variable		Sample size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-R Test
	$\bar{X}$		28.733	8.119	0.217	1.203	33.790	7.10	
	S		2.670	4.533	0.128	0.578	18.023	3.05	
Clam toxicity	$\bar{X}$ 1124.0	52	0.030	- 0.111	- 0.041	- 0.144	- 0.214	0.016	
	s 754.2		0	0.134	0.061	- 0.185	- 0.242	- 0.288*	0.425 1.652

1. Mean
2. Standard deviation
3. Simple correlation coefficient.
4. Partial correlation coefficient.
5. Multiple correlation coefficient.
6. * Significant at 5% level.

Table 7. Correlation coefficients between butter-clam toxicity levels and Phytoplankton populations for all stations combined.

A. Surface samples.

Dependent variable			Sample Size	Independent variable: Diatoms	Dinoflagellates	Gonyaulax sp.	Total cell number	R	F-Test
				[1] $\bar{X}$ 252.0	$\bar{X}$ 36.7	$\bar{X}$ 2.0	$\bar{X}$ 290.9	[5] R	
				[2] S 607.5	S 104.1	S 5.1	S 616.8		
Clam toxicity	$\bar{X}$	731.2	103	[3] -0.065	0.071	0.298**	-0.052	0.338	3.162*
	S	688.6		[4] 0	0	0.326**	0		

B. Means of four depths.

Dependent variable			Sample Size	Independent variable: Diatoms	Dinoflagellates	Gonyaulax sp.	Total cell number	R	F-Test
				$\bar{X}$ 269.8	$\bar{X}$ 35.9	$\bar{X}$ 1.5	$\bar{X}$ 308.3	R	
				S 579.4	S 115.8	S 3.3	S 588.6		
Clam toxicity	$\bar{X}$	731.2	103	-0.081	0.021	0.228*	-0.076	0.288	2.220
	S	688.6		0.118	0.114	0.254*	-0.118		

1. Mean
2. Stadard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient.
5. Multiple correlation coefficient.
6. * Significant at 5% level
   ** Significant at 1% level

Table 8. Correlation coefficients between butter-clam toxicity levels and hydrographic conditions of all stations combined.

A. Surface samples

			Independent variable						
	Sample		Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-
Dependent variable		[1] $\bar{X}$	28.291	6.875	0.220	1.086	33.420	7.45	[5] R
	Size	[2] S	2.688	4.930	0.131	0.717	18.090	3.75	Test
Clam toxicity	$\bar{X}$ 731.2	103 [3]	0.040	0.055	-0.059	-0.001	-0.122	-0.041	0.397 2.996*
	S 688.6	[4]	-0.091	0.311**	-0.119	-0.084	-0.381**	-0.114	

B. Means of four depths

			Independent variable						
	Sample		Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-
Dependent variable		$\bar{X}$	28.850	7.677	0.230	1.159	34.185	7.12	R
	Size	S	2.353	4.683	0.135	0.606	17.390	3.40	Test
Clam toxicity	$\bar{X}$ 731.2	103	0.015	0.058	-0.069	0.019	-0.109	-0.026	0.356 2.316*
	S 688.6		-0.121	0.207*	-0.130	0	-0.324**	-0.101	

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient
6. * significant at 5% level
   ** significant at 1% level

11 and 12.

Diatom numbers did not have a significant correlation with salinity, but numbers of dinoflagellates, including Gonyaulax, sp., showed a highly negative correlation with salinity, i.e., these organisms were more abundant during time of relatively low salinity(22‰ - 29‰).

As expected, phytoplankton populations, especially dinoflagellates (including Gonyaulax sp.), had a significant negative correlation with inorganic nutrients, nitrates, phosphates and silicates. Diatom blooms definitely coincided with low silicate concentrations. None of the phytoplankton populations showed any correlation with nitrite.

Both Gonyaulax sp. and other dinoflagellates (see Appendix B) had a positive relationship with temperature. These organisms were more abundant during the spring and summer. Temperature was not significantly correlated with diatom numbers.

Table 9. Correlation coefficients between phytoplankton populations and hydrographic conditions for three high-toxicity stations combined, surface samples.

Dependent variable		Sample Size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-Test
	[1]$\bar{X}$		28.220	7.372	0.204	1.123	32.280	7.37	
	[2]S		3.108	4.870	0.123	0.749	18.789	3.26	
Diatoms	$\bar{X}$ 160.2	52	[3]				-0.389**		
	S 224.3		[4] 0.556**				-0.417**	0.721	8.099**
Dinoflagellates	$\bar{X}$ 44.4	52	-0.303*	-0.477**		-0.387**	-0.431**	0.430**	
	S 113.6							0.527	2.886*
Gonyaulax sp.	$\bar{X}$ 3.2	52	-0.274*	-0.456**		-0.384**	-0.397**	0.432**	
	S 6.8							0.507	2.601*
Total cell number	$\bar{X}$ 207.4	52		-0.376**			-0.488**	0.322*	
	S 282.7		0.481**				-0.366*	0.699	7.183**

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient
6. * significant at 5% level
   ** significant at 1% level
   Correlation coefficients not significant at 1% or 5% level are not shown in the table.

Table 10. Correlation coefficients between phytoplankton populations and hydrographic conditions for three high-toxicity stations, means of four depths.

Dependent variable		Sample Size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	F-Test
	[1]$\bar{X}$		[2]$\bar{X}$ 28.733	$\bar{X}$ 8.119	$\bar{X}$ 0.217	$\bar{X}$ 1.203	$\bar{X}$ 33.790	$\bar{X}$ 7.10	[5]R
			[2]S 2.670	S 4.533	S 0.128	S 0.578	S 18.023	S 3.05	
Diatoms	$\bar{X}$ 171.2	52	[3]				-0.439**		
	S 239.8		[4] 0.404**				-0.593**	0.774	11.219**
Dinoflagellates	$\bar{X}$ 35.9	52	-0.371**	-0.541**	-0.298*	-0.523**	-0.490**	0.467**	
	S 92.6				-0.363*			0.628	4,872**
Gonyaulax sp.	$\bar{X}$ 2.1	52	-0.309*	-0.493**		-0.484**	-0.455**	0.470**	
	S 4.2				-0.313*			0.303*	0.604 4,296**
Total cell number	$\bar{X}$ 209.6	52		-0.354**		-0.322*	-0.543**		
	S 280.3		0.349*				-0.570**	0.762	10,367**

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient
6. * significant at 5% level
   ** significant at 1% level
   Correlation coefficients not significant at 1% or 5% level are not shown in the table.

Table 11. Correlation coefficients between phytoplankton populations and hydrographic conditions for all stations combined, surface samples.

Dependent variable		Sample Size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	R[5]	F-Test
	$\bar{X}$[1]		28.291	6.875	0.220	1.086	33.420	7.45		
	S[2]		2.688	4.930	0.131	0.717	18.090	3.75		
Diatoms	$\bar{X}$ 252.0	103	[3]	-0.218*		-0.203*	-0.216*			
	S 607.5		[4]	-0.209*		-0.215*		-0.407**	0.544	6.737**
Dinoflagellates	$\bar{X}$ 36.7	103	-0.289**	-0.392**		-0.353**	-0.390**	0.388**		
	S 104.1				-0.209*				0.459	4.258**
Gonyaulax sp.	$\bar{X}$ 2.0	103	-0.221*	-0.312**		-0.285**	-0.319**	0.265**		
	S 5.1								0.346	2.181
Total cell number	$\bar{X}$ 290.9	103		-0.283**		-0.262**	-0.281**			
	S 616.8			-0.207*		-0.213*		-0.384**	0.553	7.038**

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient
6. * significant at 5% level
   ** significant at 1% level
   Correlation coefficients not significant at 1% or 5% level are not shown in the table.

Table 12. Correlation coefficients between phytoplankton populations and hydrographic conditions for all stations combined, means of four depths.

Dependent variable		Sample Size	Independent variable: Salinity	Nitrates	Nitrites	Phosphates	Silicates	Temp. °C	R	F-Test
	[1]$\bar{X}$		28.850	7.677	0.230	1.159	34.185	7.12		
	[2]S		2.353	4.683	0.135	0.606	17.390	3.40		
Diatoms	$\bar{X}$ 269.8	103	[3]			-0.215*	-0.251*			
			[4]		0.233*	-0.370**	-0.316**	-0.551**	[5]0.637**	10.920**
	S 579.4									
Dinoflagellates	$\bar{X}$ 35.9	103	-0.302**	-0.406**	-0.235*	-0.399**	-0.370**	0.402**		
					-0.327**			0.241*	0.521*	5.949**
S 115.8										
Gonyaulax sp.	$\bar{X}$ 1.5	103	-0.291**	-0.383**		-0.370**	-0.367**	0.323**		
									0.424	3.508**
S 3.3										
Total cell number	$\bar{X}$ 308.3	103		-0.264**		-0.293**	-0.323**			
						-0.340**	-0.312**	-0.505**	0.624**	10.226**
S 588.6										

1. Mean
2. Standard deviation
3. Simple correlation coefficient
4. Partial correlation coefficient
5. Multiple correlation coefficient
6. * significant at 5% level
   ** significant at 1% level
   Correlation coefficients not significant at 1% or 5% level are not shown in the table.

DISCUSSION

Butter-Clam Toxicity

The physiological and death phenomena (loss of muscular coordination, gasping of air, convulsions, paralysis, and respiratory failure) demonstrated by mice following intraperitoneal injection of butter clam extracts were identical to the phenomena following injection of pure butter-clam toxin.

The fluctuations of butter-clam toxicity at the three high-toxicity stations did not follow any consistent seasonal pattern, and clams were apparently capable of accumulating significant amounts of toxin during any season of the year (e.g., peaks of toxicity appeared in alternate months at station CT 1-a and CT 1-b). Chamber and Magnusson (1950) also found that butter clams showed some degree of toxicity at every sampling, and maintained their toxicity throughout the year. These results differ from those found for California mussels (Mytilus californianus) which are only toxic for a short time during the summer (Sommer et al., 1937).

Since high butter-clam toxicity peaks occurred when phytoplankton density had been extremely low for up to three months (compare graphs of phytoplankton and toxicity

levels) an alternative source for toxicity is suggested. The three high-toxicity stations were all located within Icy Passage, and Stations CT 1-a and CT 1-b were only 1.4 nautical miles apart. Hydrographic conditions and phytoplankton (composition and density) were similar at these two stations, yet fluctuations of butter-clam toxicity levels were quite different. This suggests the possibility that a toxin source may be present in the sediment.

The U.S. Food and Drug Administration has established the maximum human tolerance for PSP as 1200 MU per 100 grams of shellfish meat, or about 2500 MU per meal (Halstead, 1965). During the investigation, only 19 out of 53 butter clam samples collected at the three high-toxicity stations were higher than 1200 MU/100 g and only 3 samples were more than 2500 MU/100 g (all from station CT 1-b). Butter clam samples taken from moderate and low-toxicity stations never exceeded these toxicity levels.

Our investigation was unable to resolve the butter-clam toxicity problem, although the striking similarities between hydrographic properties among the high-toxicity stations suggest a common denominator. Further work of a similar nature should be pursued. The following suggestions might be considered:

1. A more accurate and sensitive PSP assay method must be devised.

2. The food habits of the clam should be studied in detail to investigate the possibility that certain organisms may be responsible for butter-clam poisoning. The approach of Smith (1928) could be used. From the same collection area two groups of butter clams should be taken, one for examination of the food contents and the other for the extraction of toxin. This approach should be used at each sampling period so that any changes in toxicity can be correlated with the presence of organisms in the digestive tract of the clams.

3. Separate phytoplankton and zooplankton extracts should be made. Changes in toxicity of these extracts could then be compared with levels of toxicity in butter clams and with phytoplankton and zooplankton density. Toxicity levels found in such extracts might represent better indicators of occurrence of toxin in butter clams than hydrographic parameters or phytoplankton enumeration.

4. Since butter-clam toxicity levels were not correlated with the abundance of the Gonyaulax sp., a genus known to be a causative organism of California mussel poisoning (Sommer, et al., 1937), an intensive study of phytoplankton populations is necessary. Isolation and cultivation of representative phyoplankton species should be conducted to determine their toxicity. In addition, a source of the toxin in the sediments should be sought. Interstitial organism, zooplankton, blue-green algae, spores of the genus Gonyaulax or other kinds of dinoflagellates, or organic detritus may be sources of the toxin, and should all be investigated.

5. Burke et al., (1960) showed that the toxins extracted from butter clams, California mussels and Gonyaulax catenella all had similar chemical, physical and biological properties. In a review of the findings of the present investigation, the properties of the toxin extracted from Southeast Alaska butter clam should be analyzed again and compared to those of the toxin produced by Gonyaulax catenella.

6. There is a need to know what happens to the clams after the clams ingest toxic materials, and how they accumulate and excrete the toxin. Incorporation

of toxin by butter clam may be a result of the metabolism of the clam itself.

7. Samples should be taken weekly in the summer and biweekly in the winter, because the changes in any parameter between two consecutive sampling periods might be the key to the poisoning problem. Butter clam sample could be collected with the aid of wet suit and snorkel in the intertidal zone, if necessary.

8. Since no additional information was obtained from samples collected at depth, only surface sample should be taken in future work.

Phytoplankton Populations

The growth of phytoplankton is dependent upon solar energy and the presence of certain inorganic chemical constituents in the seawater, such as oxygen, carbonate, ammonia, nitrate, nitrite, phosphate, silicate and trace elements. Biological and physical factors that directly or indirectly affect the availability of the dissolved chemical constituents are also important in phytoplankton production. In the natural environment, the relationship between ecological factors and the growth of phytoplankton cannot be expressed in a single linear correlation, because the factors apparently react with each other, and obscure the individual effects. Even though all ecological factors are present, growth of phytoplankton may still occur at varying rates. However, when all conditions are ideal, the growth rate will be high and a bloom will occur. In the case of carbonate, the reserves of this material in

seawater, in the air, and in rocks and sediments are so great that the disturbance of the carbonate balance in seawater by phytoplankton activity appears to be negligible. On the other hand, the concentration of ammonia, nitrate, nitrite, phosphate and silicate in seawater is sufficiently small, relatively speaking, that phytoplankton disturbance is very evident. Thus, vigorous phytoplankton activity may lead to depletion of these nutrients during the growth season. After maximum production is reached, the phytoplankton population decreases until further regeneration of nutrients has taken place to reach a level high enough to support another bloom. The results of this investigation showed a negative correlation between the major groups of phytoplankton population and inorganic nutrients, nitrate, phosphate and silicate, i.e. a bloom caused a decrease of inorganic nutrient concentrations. In coastal areas where upwelling is seasonal or periodic, the nutrient content of the surface waters may show marked fluctuations and may actually increase during the season of maximum phytoplankton activity (Sverdrup, et al., 1942). Similar phenomena were observed at stations CT 7, CT 109, and CT 8, and the fall diatom bloom of 1968 did not result in decreased nitrate, phosphate, and silicate concentrations. From this, it is obvious that seasonal fluctuations of a nutrient can not be ascribed to biological processes alone.

All three inorganic nitrogen compounds, ammonia, nitrate and nitrite, can be utilized by phytoplankton, or at least by some species. Nitrite can be absorbed directly only under certain circumstances. Harvey (1953) proved that nitrite cannot be used in the dark. Nitrite is only a transitional stage in the regeneration of nitrate and is not as abundant as ammonia and nitrate, so that it is usually considered that the nitrate and ammonia are the important forms of inorganic nitrogen. The fact that nitrite level is not significantly correlated with phytoplankton populations during this investigation may be due to the above. The decrease of nitrite concentration in the fall and winter could be caused by mixing of surface with bottom water, where the nitrite concentration is lower than the surface water and is mostly oxidized to nitrate by nitrifying bacteria. Harvey (1957) found that water close to the bottom is a site of active nitrification, particularly in water of moderate depth. However, nitrification may also occur within the water column.

Phosphate is also considered a primary nutrient of phytoplankton. The utilization of phosphate in the synthesis of organic substances proceeds at an approximately parallel rate to that of nitrate (Svedrup et al., 1942). Silicon-dioxide is known to be an essential part of the solid structure of silicoflagellates and of diatoms. Thus,

silicate is a biologically significant element, and like phosphate and nitrate, it exhibits a seasonal variation (Sverdrup et al., 1942). The results of the present study showed that phosphate and silicate had a negative correlation with phytoplankton populations and low silicate concentrations definitely coincided with diatom blooms.

Braarud (1935) found that dinoflagellate maxima may often be correlated with the seasonal occurrence of relatively low salinity. Ryther (1955) classified the great majority of dinoflagellates as warm-water organisms, which is particularly true if they are compared as a group with the diatoms, which in contrast, are often considered cold-water forms. Similar results were found in this study; dinoflagellate numbers were negatively correlated with salinity and positively correlated with temperature. The maxima of dinoflagellates were observed at relatively low (22‰ - 29‰) salinity and relatively high temperature (7°C - 16°C).

The flowering of dinoflagellates tended to occur a little later than the diatom bloom. The basic reason for this type of succession may be nutritional, although the exact mechanism is obscure. The diatom blooms may reduce the concentrations of nutrients to levels favorable for the growth of dinoflagellates (Hutchinson, 1944), or the diatoms may produce some external metabolites which may facilitate the growth of dinoflagellates and inhibit the growth of

other competing phytoplankton (Lucas, 1949). There was no flowering of dinoflagellates after the 1968 fall bloom of diatoms at stations CT 7, CT 109 and CT 8. This may have been due to the temperature, which was below the optimal range and could have inhibited the growth of the dinoflagellates. Gonyaulax sp., an armoured dinoflagellate, had a relationship to hydrographic conditions similar to that of the rest of the dinoflagellate populations.

All samples were collected from shallow water with the deepest sample from 38 meters. The vertical distribution of inorganic nutrient concentrations did not change markedly with depth, and variations were especially small during the winter and fall. Since samples were collected within the euphotic zone, the vertical distribution of phytoplankton was not significantly affected by the availability of either sunlight or inorganic nutrients, so that the variations were small and were negligible for the purpose of this investigation (compare the graphs of the surface sample and the mean).

The complexity and disagreement about the classification of Gonyaulax made it difficult to positively identify this organism. A photograph of Gonyaulax sp. from the phytoplankton sample collected at Station CT 1-b, on 11 July 1969, is shown in Figure 23 and 24. Figure 25 is the same species

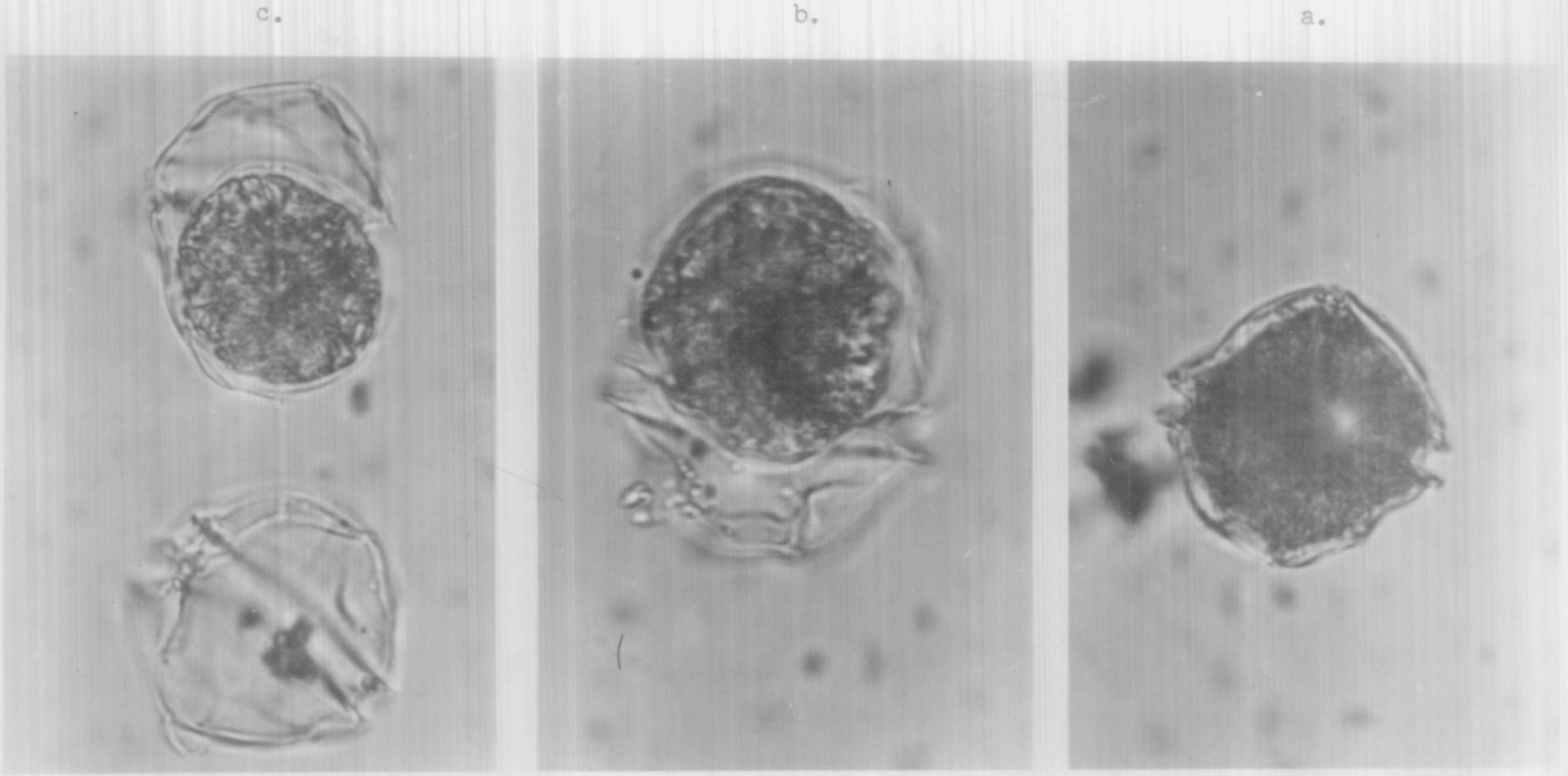
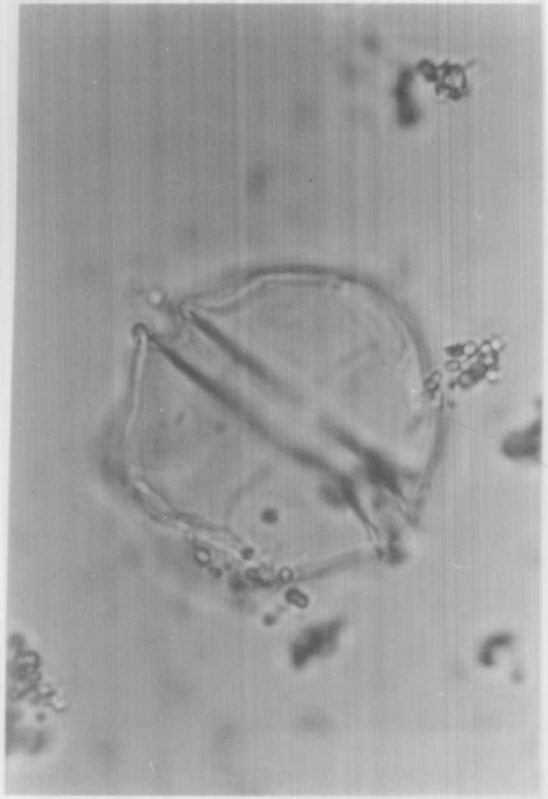


Figure 23. Photomicrographs of microorganisms tentatively identified as Gonyaulax sp. from station CT 1-b. Magnification: 12.5 x 40.

a. Diameter, 27.5 µm. b. Diameter, 37.0 µm. c. Diameter of the cell, 29.5 µm, diameter of the theca, 33.0 µm.

b.



a.

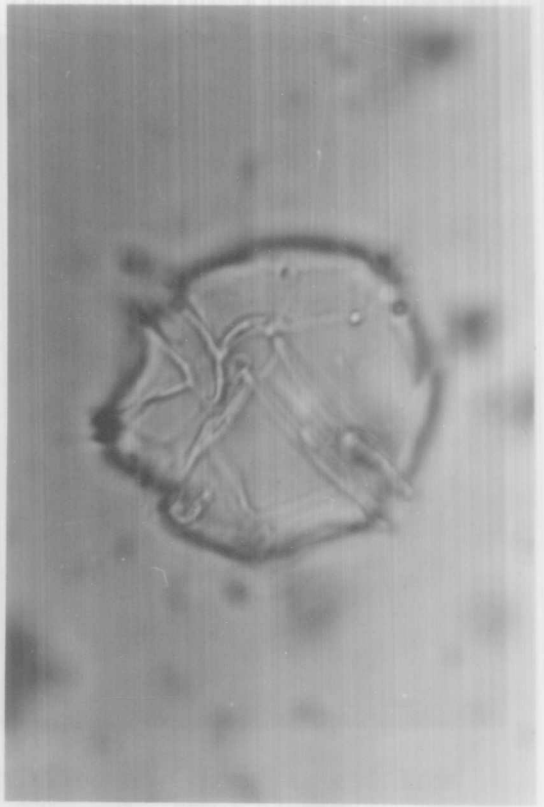
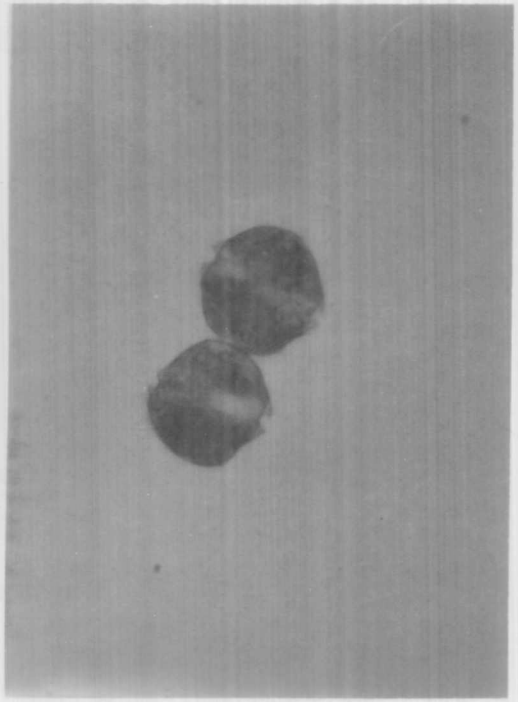


Figure 24. Photomicrographs of the theca of microorganisms tentatively identified as Gonyaulax sp. from station CT 1-b. Magnification: 12.5 x 40.

a. Diameter, 40.5 µm. b. Diameter, 42.5 µm.

a.



b.

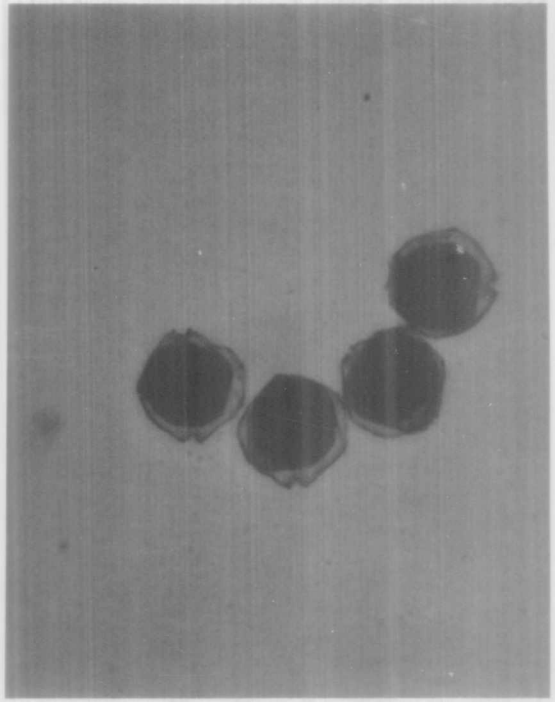


Figure 25. Photomicrographs of microorganisms tentatively identified as Gonyaulax sp. from pure culture. Magnification: 10 x 40.

a. Living cells, diameter, 16.8 μm. b. Cells fixed by modified Lugol solution*, diameter, 16.8 μm.

* Modified Lugol solution ingredients: 10 g I_2 , 20 g KI, 20 ml acetic acid, 50 ml distilled water.

grown in pure culture (Erd Schreiber medium*) by Dr. K. V. Natarajan (unpublished results). The picture was taken one week after inoculation. The diameter of the Gonyaulax sp. from pure culture is about 16 to 18 μm, which is much smaller than the one from the natural environment (about 25 to 55 μm). A chain composed of 2 to 4 cells was observed in pure culture during the period when the picture was taken. No chain form was found in the natural environment.

Gonyaulax catenella is the causative organism of California mussel poisoning (Sommer et al., 1937), but no consistently significant correlation was found between this genus and butter-clam toxicity levels in Southeast Alaska. Foster and Sommer (Schantz and Magnusson, 1964) were also unable to relate the occurrence of Gonyaulax sp. to changes of toxicity levels in the butter clam.

*Erd Schreiber medium contents:

Icy Strait natural seawater	1000.0	ml
$NaNO_3$	0.2	g
Na_2HPO_4 $12H_2$)	0.03	g
Soil extract	50.0	ml

SUMMARY

1. Seasonal variations of butter-clam toxicity levels did not follow a similar pattern at all stations. No consistent pattern was observed. At three high-toxicity stations the butter clams occasionally accumulated significant amounts (higher than the maximum human tolerance) of paralytic shellfish poison (PSP) during any season from August 1968 to October 1969, and occasionally lost or regained PSP very rapidly between two samplings.

2. During the investigation, only 19 out of 53 samples collected at the three high-toxicity stations were higher than the maximum human tolerance PSP--1200 MU--established by U.S. Food and Drug Administration, and the clam samples taken from moderate and low-toxicity stations never exceeded this toxicity level.

3. Two patterns of diatom fluctuation were observed. The diatom variations at the four most toxic stations were similar. The diatom populations were extremely low during the fall and winter and the highest peak in the most toxic stations occurred in late spring. The three low-toxicity stations followed another pattern. The highest peaks occurred in mid-fall and early-spring.

4. The maxima for dinoflagellate numbers and Gonyaulax

sp. occurred mainly in spring and summer. At other times of the year these populations were extremely low.

5. The dinoflagellate peak occurred a little later than the peak of diatoms.

6. The dinoflagellate population density was considerably less than that of the diatom populations.

7. A species tentatively identified as Gonyaulax was found in the phytoplankton but only represented a minority of the dinoflagellate population.

8. The annual pattern of the changes in salinity, inorganic nutrients and water temperature was similar at all stations.

9. The salinity and concentrations of nitrate, phosphate and silicate were relatively high with small variations during the winter and relatively low with large variations during the summer.

10. Nitrite was most abundant in the fall after the period of the most rapid utilization of nitrate by phytoplankton. Nitrite then decreased to a minimum at approximately the period of maximum nitrate. The nitrite never reached a concentration as high as nitrate and the changes were not as marked.

11. In general, water temperatures were similar at all stations. Their seasonal distribution followed a consistent pattern. Minimum temperatures were 0.8°C to

3.3°C from January to March, slowly increasing to maximum temperatures of 7.5°C to 18°C from June to September.

12. Vertical distributions of phytoplankton populations and hydrographic conditions were quite uniform with depth; the variations were especially small during the fall and winter. No additional information was obtained from the results of four depths. The graph of the mean and the surface is basically similar.

13. Neither phytoplankton populations nor hydrographic parameters had a consistently significant relationship with butter-clam toxicity levels.

14. A small number of Gonyaulax sp. was found in the phytoplankton samples. This genus is the causative organism of California mussel poisoning, but its occurrence did not coincide with butter-clam toxicity levels.

15. At three high-toxicity stations several high-toxicity peaks occurred at times when populations of phytoplankton had been extremely low for up to three months. The three high-toxicity stations were all within Icy Passage, and their fluctuations of phytoplankton populations and hydrographic conditions were similar, yet the fluctuations of toxicity levels were quite different. These two facts suggest the possibility that the butter clam in Southeast Alaska may have source of toxic materials other than the phytoplankton--Gonyaulax sp.

16. A series of suggested approaches for future work on the butter-clam poisoning problem is presented. These approaches emphasize on the design of a new PSP assay method, and studies of food habits and metabolism of butter clams. The search for new causative materials for butter-clam toxicity, and the analysis of the chemical and physical properties of the saxitoxin is recommended.

17. The changes in phytoplankton populations have a significantly negative correlation with the changes in inorganic nutrients, nitrate, phosphate, and silicate. Low silicate concentrations definitely coincide with diatom blooms.

18. The dinoflagellate numbers were negatively correlated with salinity and positively correlated with temperature, i.e. dinoflagellate blooms tended to occur at relatively low salinity (22‰ - 29‰) and relatively high temperatures ($7^{\circ}C$ - $16^{\circ}C$). Diatoms were not significantly related to either salinity or temperature.

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APPENDIX A

SOMMER'S TABLE

Death Time-Mouse Unit Relations for Paralytic Shellfish Poison (from Halstead, 1965)

Death Time Min:sec	Mouse Units	Death Time Min:sec	Mouse Units	Death Time Min:sec	Mouse Units	Death Time Min:sec	Mouse Units
1:08	100	3:00	3.70	4:55	1.96	9:30	1.13
10	66.2	05	3.57	5:00	1.92	10:00	1.11
15	38.3	10	3.43	05	1.89	30	1.09
20	26.4	15	3.31	10	1.86	11:00	1.075
25	20.7	20	3.19	15	1.83	30	1.06
30	16.5	25	3.08	20	1.80	12:00	1.05
35	13.9	30	2.98	30	1.74	13	1.03
40	11.9	35	2.88	40	1.69	14	1.015
45	10.4	40	2.79	45	1.67	15	1.000
50	9.33	45	2.71	50	1.64	16	0.99
55	8.42	50	2.63	6:00	1.60	17	0.98
2:00	7.67	55	2.56	15	1.54	18	0.972
05	7.04	4:00	2.50	30	1.48	19	0.965
10	6.52	05	2.44	45	1.43	20	0.96
15	6.06	10	2.38	7:00	1.39	21	0.954
20	5.66	15	2.32	15	1.35	22	0.948
25	5.32	20	2.26	30	1.31	23	0.942
30	5.00	25	2.21	45	1.28	24	0.937
35	4.73	30	2.16	8:00	1.25	25	0.934
40	4.48	35	2.12	15	1.22	30	0.917
45	4.26	40	2.08	30	1.20	40	0.898
50	4.06	45	2.04	45	1.18	60	0.875
55	3.88	50	2.00	9:00	1.16		

Weight Correction Factors for Mouse of PSP Bioassay

Wt. of Mice(g)	Mouse Units	Wt. of Mice(g)	Mouse Units	Wt. of Mice(g)	Mouse Units	Wt. of Mice(g)	Mouse Units
10	0.50	14½	0.76	19	0.97	23	1.07
10½	0.53	15	0.785	19½	0.985	23½	1.075
11	0.56	15½	0.81	20	1.000	24	1.08
11½	0.59	16	0.84	20½	1.015	24½	1.085
12	0.62	16½	0.86	21	1.03	25	1.09
12½	0.65	17	0.88	21½	1.04	26/27	1.10
13	0.675	17½	0.905	22	1.05	28/29	1.11
13½	0.70	18	0.93	22½	1.06	30	1.12
14	0.73	18½	0.95				

APPENDIX B

Selected counts of major phytoplankton genera (in cells/ml), upper numbers are surface samples and lower numbers are means of four depths.

STA. CT 1a Sampling Date	1968 Aug. 24	Sept. 22	Oct. 23	Nov. 19	Dec. 19	1969 Jan. 16	Feb. 13
Total no. of diatoms	45.5	18.5	1.9	3.3	0.9	1.6	2.7
	19.9	16.9	1.8	2.7	0.5	1.7	1.7
Chaetoceros	29.4	0.0	0.0	0.5	0.0	0.0	0.0
	11.6	10.3	0.0	0.2	0.0	0.4	0.0
Detonula	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	6.7	0.7	0.0	0.0	0.0	0.0	0.0
	2.2	0.2	0.0	0.0	0.0	0.1	0.0
Navicula	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitzschia	3.6	0.0	0.0	0.2	0.0	0.0	1.5
	1.2	0.0	0.0	0.3	0.1	0.4	0.4
Skeletonema	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.7	0.0	0.0	0.0	0.0	0.0	0.0
Thalassiosira	1.0	13.7	0.3	0.7	0.6	1.0	0.8
	1.0	4.6	0.8	0.9	0.2	0.7	0.8
Total no. of dinoflagellates	0.8	61.7	0.9	0.0	0.0	0.0	0.8
	0.5	23.5	1.7	0.0	0.0	0.0	0.6
Unidentified unarmoured dinoflagellates	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gonyaulax	0.0	6.5	0.9	0.0	0.0	0.0	0.8
	0.2	2.2	1.6	0.0	0.0	0.0	0.6
Peridinium	0.8	54.5	0.0	0.0	0.0	0.0	0.0
	0.3	21.1	0.0	0.0	0.0	0.0	0.0
Grand total cell no.	46.3	80.9	2.8	3.7	0.9	1.6	3.5
	20.3	40.9	3.5	3.4	0.5	1.7	2.3

STA. CT 1a Sampling Date	1969 Mar. 19	Apr. 16	May 13	June 12	June 30	July 11
Total no. of diatoms	289.8 326.5	304.9 409.8	266.5 371.7	211.5 417.8	610.2 538.1	303.4 458.0
Chaetoceros	176.4 209.7	44.6 125.1	0.0 6.3	83.3 125.3	0.0 1.3	0.0 2.9
Detonula	4.7 5.9	11.2 8.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.0 0.0	0.0 0.0	0.0 0.0	0.0 4.4	483.4 423.4	0.0 13.8
Navicula	6.3 2.8	0.0 0.3	5.8 2.1	2.5 4.7	0.0 2.5	0.0 11.3
Nitzschia	43.0 33.0	5.5 11.9	2.5 7.5	30.8 59.6	88.4 72.5	21.7 33.0
Skeletonema	13.8 16.3	0.0 3.5	0.0 0.0	0.0 0.0	0.0 0.0	205.0 227.1
Thalassiosira	43.7 58.5	243.6 261.1	258.2 352.6	89.1 217.0	0.0 5.4	5.0 7.1
Total no. of dinoflagellates	1.2 0.5	1.3 0.7	14.1 20.2	8.3 6.2	71.7 74.6	576.3 480.6
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	8.3 18.7	0.0 0.0	55.0 59.6	515.8 447.6
Gonyaulax	0.9 0.5	0.0 0.0	0.0 0.0	0.0 0.0	5.0 6.7	21.7 16.7
Peridinium	0.3 0.1	1.3 0.7	5.8 1.5	8.3 6.2	11.7 8.4	38.3 16.3
Grand total cell no.	291.0 327.1	306.2 410.3	280.6 391.9	219.8 424.0	681.9 612.7	880.2 938.6

STA. CT 1a	Sampling Date	1969 July 27	Aug. 15	Aug. 27	Sept. 27	Oct. 23
Total no. of diatoms		38.0	64.5	99.2	8.9	336.7
		73.9	52.9	53.6	31.3	306.7
Chaetoceros		0.0	18.8	43.4	0.0	0.8
		2.1	12.5	20.7	0.0	1.4
Detonula		0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0	0.0
Leptocylindrus		0.0	0.0	0.0	0.0	0.0
		5.0	0.0	0.0	0.0	0.0
Navicula		0.0	0.0	0.8	0.0	1.8
		0.0	1.6	0.4	0.0	1.1
Nitzschia		8.5	12.5	5.0	2.6	32.6
		10.9	4.7	1.7	15.1	27.0
Skeletonema		0.0	0.0	17.5	0.0	273.3
		0.0	0.0	5.8	1.9	255.6
Thalassiosira		5.0	14.4	15.0	6.3	23.3
		7.4	10.5	9.2	14.0	19.1
Total no. of dino-flagellates		20.0	53.8	27.6	0.0	0.8
		30.8	60.5	20.5	1.4	0.3
Unidentified unarmoured dinoflagellates		0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0	0.0
Gonyaulax		0.0	0.0	9.3	0.0	0.0
		0.0	0.0	7.2	0.8	0.0
Peridinium		0.0	22.5	10.8	0.0	0.8
		0.0	24.4	7.1	0.7	0.3
Grand total cell no.		68.0	139.0	136.9	8.9	337.5
		119.3	133.8	83.2	33.8	307.5

STA. CT 1b Sampling Date	1968 Sept. 22	Oct. 23	Nov. 19	Dec. 19	1969 Jan. 16	Feb. 13	Mar. 19
Total no. of diatoms	38.7	2.9	2.0	2.5	1.0	0.8	335.7
	35.5	3.1	1.8	1.4	2.0	1.4	303.0
Chaetoceros	5.8	0.0	0.0	0.0	0.0	0.0	184.5
	14.5	0.0	0.0	0.0	0.4	0.0	162.1
Detonula	0.7	0.3	0.0	0.0	0.0	0.0	13.9
	1.0	0.2	0.0	0.0	0.0	0.0	7.8
Leptocylindrus	0.0	0.0	0.0	0.3	0.5	0.0	0.0
	4.2	0.1	0.0	0.2	0.3	0.0	0.0
Navicula	0.0	0.0	0.0	0.0	0.0	0.0	4.2
	0.0	0.0	0.0	0.0	0.0	0.0	2.3
Nitzschia	4.1	0.0	0.5	0.0	0.5	0.0	37.5
	1.9	0.0	0.3	0.1	0.4	0.1	43.4
Skeletonema	14.4	0.0	0.0	0.0	0.0	0.0	26.4
	6.2	0.0	0.0	0.0	0.0	0.0	21.7
Thalassiosira	6.5	0.5	0.7	2.2	0.0	0.8	68.6
	4.3	1.6	0.6	0.9	0.1	1.2	65.5
Total no. of dinoflagellates	23.7	2.8	0.0	0.3	0.0	0.8	2.1
	12.2	2.3	0.1	0.1	0.0	0.4	1.4
Unidentified unarmoured dinoflagellates	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gonyaulax	4.8	2.8	0.0	0.3	0.0	0.8	0.6
	2.2	2.1	0.0	0.1	0.0	0.4	0.6
Peridinium	16.8	0.0	0.0	0.0	0.0	0.0	1.5
	9.4	0.0	0.0	0.0	0.0	0.0	0.4
Grand total cell no.	63.8	5.7	3.4	2.8	1.0	1.6	337.8
	48.2	5.4	3.1	1.5	2.0	1.8	304.5

STA. CT 1b Sampling Date	1969 Apr. 16	May 13	June 12	June 30	July 11	July 27
Total no. of diatoms	335.3	322.3	792.8	466.8	683.6	55.0
	548.3	355.3	956.5	348.4	501.4	68.6
Chaetoceros	107.2	0.0	280.1	0.0	0.0	8.5
	219.1	0.0	221.0	0.0	0.0	8.0
Detonula	0.0	0.0	0.0	0.0	0.0	0.0
	1.8	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	0.0	378.4	11.7	6.5
	0.0	0.0	0.0	243.4	7.1	1.6
Navicula	0.0	2.5	8.8	0.0	0.0	0.0
	0.3	1.3	4.1	0.0	1.3	0.0
Nitzschia	5.4	5.8	103.8	61.7	26.7	8.5
	9.4	3.5	115.7	64.6	23.0	10.4
Skeletonema	4.2	0.0	0.0	0.0	516.8	8.5
	1.1	0.0	3.1	0.0	366.3	2.1
Thalassiosira	218.5	314.0	396.3	0.0	0.0	3.5
	309.4	349.9	604.4	7.1	1.3	4.3
Total no. of dinoflagellates	2.9	30.0	3.8	116.7	351.7	73.0
	1.7	31.0	3.8	60.0	388.0	63.3
Unidentified unarmoured dinoflagellates	0.0	25.0	3.8	66.7	278.4	0.0
	0.0	28.5	1.0	30.9	344.7	0.0
Gonyaulax	0.0	2.5	0.0	21.7	33.3	0.0
	0.5	0.6	1.0	7.9	19.6	0.0
Peridinium	2.9	2.5	0.0	28.3	28.3	1.5
	1.2	1.9	1.8	21.2	18.3	0.8
Grand total cell no.	338.2	352.3	796.6	583.5	1035.3	136.0
	550.0	386.3	960.3	408.4	889.4	139.1

STA. CT 1b Sampling Date	1969 Aug. 15	Aug. 27	Sept. 28	Oct. 23
Total no. of diatoms	10.1 16.0	63.8 58.6	43.3 15.8	105.0 150.8
Chaetoceros	0.0 0.8	38.6 23.0	0.0 0.0	0.0 0.2
Detonula	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Navicula	0.0 0.3	0.0 0.4	0.0 0.3	0.8 0.4
Nitzschia	0.8 1.0	1.8 1.9	12.0 4.0	20.1 25.2
Skeletonema	0.0 0.2	0.0 1.5	0.0 0.0	67.5 110.3
Thalassiosira	2.5 4.5	9.3 13.1	29.7 11.1	13.3 12.7
Total no. of dinoflagellates	11.6 14.8	9.4 10.7	10.5 6.3	0.8 0.4
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	0.0 0.0	3.3 3.7	6.8 3.9	0.0 0.2
Peridinium	5.8 5.0	1.8 3.6	3.7 1.3	0.8 0.2
Grand total cell no.	22.5 38.6	79.0 80.0	75.2 29.9	106,6 152.1

STA. CT 2 Sampling Date	1968 Aug. 24	Sept. 22	Oct. 23	Nov. 19	Dec. 19	1969 Jan. 16	Feb. 13
Total no. of diatoms	84.7		4.4	2.3	0.6	0.5	0.8
	84.7		5.7	2.5	0.9	2.0	3.1
Chaetoceros	72.3		0.0	0.0	0.0	0.0	0.0
	72.3		0.0	0.0	0.0	0.7	0.1
Detonula	0.0		1.4	0.0	0.0	0.0	0.0
	0.0		1.4	0.0	0.0	0.0	0.0
Leptocylindrus	1.3		0.0	0.0	0.0	0.0	0.4
	1.3		0.1	0.0	0.0	0.3	0.2
Navicula	0.1		0.3	0.0	0.0	0.0	0.0
	0.1		0.1	0.0	0.0	0.0	0.0
Nitzschia	2.0		0.0	0.0	0.0	0.0	0.0
	2.0		0.4	0.2	0.2	0.4	0.1
Skeletonema	2.6		0.0	0.0	0.0	0.0	0.0
	2.6		0.7	0.0	0.0	0.0	0.0
Thalassiosira	3.8		0.0	0.7	0.0	0.0	0.4
	3.8		0.5	0.6	0.3	0.3	1.5
Total no. of dinoflagellates	1.5		1.0	0.0	0.0	0.0	0.0
	1.5		1.0	0.0	0.0	0.1	0.0
Unidentified unarmoured dinoflagellates	0.0		0.0	0.0	0.0	0.0	0.0
	0.0		0.0	0.0	0.0	0.0	0.0
Gonyaulax	0.5		1.0	0.0	0.0	0.0	0.0
	0.5		0.9	0.0	0.0	0.0	0.0
Peridinium	0.9		0.0	0.0	0.0	0.0	0.0
	0.9		0.1	0.0	0.0	0.1	0.0
Grand total cell no.	86.2		5.4	4.2	0.6	0.5	0.8
	86.2		6.7	3.3	1.0	2.2	3.1

STA. CT 2 Sampling Date	1969 Mar. 19	Apr. 16	May 13	June 13	June 30	July 11	July 27
Total no. of diatoms	97.5 67.1	144.7 215.3	70.0 54.0	837.7 1032.8	720.1 438.9	270.1 304.2	70.0 60.6
Chaetoceros	42.1 22.7	45.9 76.2	0.0 0.0	300.0 351.3	0.0 2.9	0.0 1.3	0.0 0.0
Detonula	7.3 2.3	0.0 1.5	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.0 0.0	0.0 0.0	0.0 0.0	3.8 1.0	423.6 300.5	0.0 4.2	0.0 0.0
Navicula	1.1 2.4	7.1 3.9	7.0 2.5	0.0 3.2	0.0 2.9	5.0 1.3	0.0 0.0
Nitzschia	14.1 8.3	8.3 5.9	3.0 2.3	83.8 147.3	38.3 32.9	38.4 53.8	5.0 5.5
Skeletonema	19.8 9.9	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	116.7 163.8	0.0 0.0
Thalassiosira	12.1 20.9	83.4 126.9	57.0 47.8	428.8 519.7	5.0 12.5	5.0 5.4	6.5 4.8
Total no. of dinoflagellates	0.0 0.0	0.0 0.0	56.0 40.8	0.0 0.0	173.4 96.7	478.4 285.1	85.0 96.8
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	53.0 37.5	0.0 0.0	128.4 71.3	433.4 250.1	0.0 0.0
Gonyaulax	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	16.7 11.3	16.7 9.6	0.0 0.0
Peridinium	0.0 0.0	0.0 0.0	3.0 3.3	0.0 0.0	28.3 14.2	28.3 21.3	3.5 1.6
Grand total cell no.	97.5 67.1	144.7 215.3	126.0 94.8	837.7 1032.8	893.5 535.6	748.5 590.6	165.0 161.4

STA. CT 2 Sampling Date	1969 Aug. 15	Aug. 27	Sept. 28	Oct. 23
Total no. of diatoms	24.6 26.6	28.7 57.4	43.7 32.7	61.7 61.7
Chaetoceros	0.0 3.0	4.3 14.5	0.0 0.3	0.0 0.0
Detonula	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Navicula	0.8 2.6	0.0 0.4	1.3 2.4	1.7 1.7
Nitzschia	2.6 2.4	2.6 1.7	15.7 10.1	9.7 9.7
Skeletonema	0.0 0.0	0.0 5.8	0.0 0.0	40.4 40.4
Thalassiosira	11.8 9.0	7.5 10.7	25.4 16.8	8.8 8.8
Total no. of dinoflagellates	20.2 13.9	5.1 5.6	8.5 5.4	0.0 0.0
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	0.0 0.0	1.8 1.1	7.2 4.3	0.0 0.0
Peridinium	3.3 2.9	0.8 1.9	1.3 1.1	0.0 0.0
Grand total cell no.	54.8 47.8	45.4 74.9	33.2 60.8	62.5 62.5

STA. CT 5 Sampling Date	1968 Aug. 25	Sept. 22	Oct. 22	Nov. 19	Dec. 18	1969 Jan. 17	Feb. 13
Total no. of diatoms	9.0 13.2	26.8 9.5	10.7 10.0	2.8 3.2	0.0 0.3	2.5 1.1	1.2 1.0
Chaetoceros	7.4 6.0	1.6 1.0	0.0 0.4	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.2
Detonula	0.0 0.0	0.5 0.1	0.3 0.7	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.1 0.1	2.6 0.7	0.0 0.2	0.0 0.0	0.0 0.0	0.0 0.0	0.4 0.1
Navicula	0.0 0.0	0.5 0.2	0.0 0.2	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Nitzschia	0.3 0.1	0.0 0.2	0.3 0.1	0.7 0.5	0.0 0.0	0.5 0.4	0.0 0.0
Skeletonema	0.0 2.3	0.0 0.0	0.0 0.5	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Thalassiosira	0.3 3.3	5.6 2.6	0.0 0.9	0.0 0.2	0.0 0.1	0.0 0.0	0.0 0.3
Total no. of dinoflagellates	0.5 0.4	6.7 2.6	0.3 0.4	0.2 0.1	0.0 0.0	0.0 0.0	0.0 0.0
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	0.0 0.1	2.6 1.4	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Peridinium	0.5 0.3	3.0 0.9	0.3 0.4	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Grand total cell no.	9.5 13.8	40.6 14.3	11.3 10.4	3.0 3.3	0.0 0.3	2.5 1.1	1.2 1.0

STA. CT 5 Sampling Date	1969 Mar. 18	Apr. 17	May 14	June 13	July 2	July 25	Aug. 14
Total no. of diatoms	38.2	90.1	35.5	1028.9	83.4	150.7	20.2
	36.5	192.6	34.4	749.9	60.6	163.1	42.9
Chaetoceros	3.2	0.0	19.2	291.3	0.0	0.0	3.3
	2.6	18.4	15.5	150.7	3.2	0.0	14.9
Detonula	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Navicula	14.8	17.9	7.1	16.3	61.7	0.0	4.3
	12.4	14.6	4.6	8.2	18.6	0.0	5.9
Nitzschia	1.6	5.5	0.0	21.3	11.7	1.1	3.3
	1.3	4.9	0.5	23.2	19.6	3.5	2.9
Skeletonema	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	1.4	0.0	5.3	0.0	0.7	2.7
Thalassiosira	10.0	63.8	7.1	512.5	5.0	8.6	4.3
	13.4	151.3	12.3	479.1	12.6	13.5	6.7
Total no. of dinoflagellates	3.5	0.0	9.3	0.0	33.3	23.6	5.8
	1.3	0.3	8.8	1.0	32.1	16.8	8.1
Unidentified unarmoured dinoflagellates	0.0	0.0	9.3	0.0	33.3	0.0	0.0
	0.0	0.0	8.8	0.0	32.1	0.0	0.0
Gonyaulax	3.5	0.0	0.0	0.0	0.0	0.0	0.0
	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Peridinium	0.0	0.0	0.0	0.0	0.0	0.0	2.5
	0.0	0.3	0.0	1.0	0.0	0.3	1.1
Grand total cell no.	42.4	90.1	44.8	1028.9	116.7	176.9	31.0
	38.0	192.9	43.3	750.9	92.7	183.5	61.6

STA. CT 7 Sampling Date	1968 Aug. 28	Sept. 21	Oct. 22	Nov. 20	Dec. 17	1969 Jan. 15	Feb. 11
Total no. of diatoms	39.8 34.0	3.3 3.3	162.8 184.8	434.5 508.1	3.6 2.9	2.3 1.0	3.2 1.4
Chaetoceros	30.4 22.3	0.0 0.0	115.7 143.4	6.9 3.7	0.3 0.3	0.0 0.0	0.0 0.0
Detonula	0.0 0.0	0.5 0.5	0.0 0.0	0.0 0.0	0.0 9.0	0.0 0.0	0.0 0.0
Leptocylindrus	2.0 0.6	0.0 0.1	1.5 1.0	1.7 1.7	0.0 0.2	0.0 0.0	0.4 0.2
Navicula	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Nitzschia	0.3 0.3	0.3 0.3	30.2 26.2	399.4 480.2	2.1 1.2	0.5 0.2	0.0 0.0
Skeletonema	4.4 7.2	0.5 0.5	0.0 0.3	11.1 8.5	0.0 0.0	0.0 0.0	0.0 0.0
Thalassiosira	1.8 2.1	0.1 0.1	11.3 8.3	10.2 7.3	0.0 0.6	0.9 0.3	1.2 0.6
Total no. of dinoflagellates	8.9 5.2	0.5 0.5	1.5 1.4	0.9 1.1	0.3 0.2	0.0 0.0	0.0 0.0
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	6.9 3.8	0.4 0.4	1.5 1.0	0.0 0.0	0.3 0.2	0.0 0.0	0.0 0.0
Peridinium	2.0 1.4	0.1 0.1	0.0 0.1	0.9 1.1	0.0 0.0	0.0 0.0	0.0 0.0
Grand total cell no.	48.7 39.4	4.9 4.9	165.8 188.8	435.4 509.2	3.9 3.1	2.3 1.0	3.2 1.4

STA. CT 7 Sampling Date	1968 Aug. 28	Sept. 21	Oct. 22	Nov. 20	Dec. 17	1969 Jan. 15	Feb. 11
Total no. of diatoms	39.8 34.0	3.3 3.3	162.8 184.8	434.5 508.1	3.6 2.9	2.3 1.0	3.2 1.4
Chaetoceros	30.4 22.3	0.0 0.0	115.7 143.4	6.9 3.7	0.3 0.3	0.0 0.0	0.0 0.0
Detonula	0.0 0.0	0.5 0.5	0.0 0.0	0.0 0.0	0.0 9.0	0.0 0.0	0.0 0.0
Leptocylindrus	2.0 0.6	0.0 0.1	1.5 1.0	1.7 1.7	0.0 0.2	0.0 0.0	0.4 0.2
Navicula	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Nitzschia	0.3 0.3	0.3 0.3	30.2 26.2	399.4 480.2	2.1 1.2	0.5 0.2	0.0 0.0
Skeletonema	4.4 7.2	0.5 0.5	0.0 0.3	11.1 8.5	0.0 0.0	0.0 0.0	0.0 0.0
Thalassiosira	1.8 2.1	0.1 0.1	11.3 8.3	10.2 7.3	0.0 0.6	0.9 0.3	1.2 0.6
Total no. of dinoflagellates	8.9 5.2	0.5 0.5	1.5 1.4	0.9 1.1	0.3 0.2	0.0 0.0	0.0 0.0
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	6.9 3.8	0.4 0.4	1.5 1.0	0.0 0.0	0.3 0.2	0.0 0.0	0.0 0.0
Peridinium	2.0 1.4	0.1 0.1	0.0 0.1	0.9 1.1	0.0 0.0	0.0 0.0	0.0 0.0
Grand total cell no.	48.7 39.4	4.9 4.9	165.8 188.8	435.4 509.2	3.9 3.1	2.3 1.0	3.2 1.4

STA. CT 7 Sampling Date	1969 Mar. 17	Apr. 18	May 14	June 14	July 1	July 26	Aug. 13
Total no. of diatoms	12.9	4721.7	126.7	67.5	185.3	63.0	15.7
	12.9	3557.9	345.9	125.2	111.1	223.5	15.6
Chaetoceros	0.0	316.8	5.0	0.0	0.0	0.0	5.7
	0.4	192.6	191.4	1.0	0.0	4.1	3.6
Detonula	0.0	3767.4	0.0	0.0	0.0	0.0	0.0
	0.0	2723.2	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	0.0	0.0	0.0	16.5	0.0
	0.0	0.0	0.0	0.0	11.3	10.4	0.0
Navicula	6.9	5.0	0.0	0.0	68.7	0.0	5.7
	6.5	6.4	1.3	1.9	32.6	0.0	5.0
Nitzschia	3.2	78.4	50.0	42.5	47.9	5.0	0.0
	2.3	84.3	59.0	47.3	20.3	5.9	1.0
Skeletonema	0.0	171.7	0.0	0.0	0.0	0.0	0.0
	0.0	112.4	0.0	0.0	0.0	0.0	0.0
Thalassiosira	0.9	310.1	71.7	25.0	0.0	5.0	1.0
	1.2	348.4	85.6	66.9	0.0	6.3	0.9
Total no. of dinoflagellates	2.5	0.0	33.3	32.5	152.1	38.5	6.7
	1.4	0.0	13.8	49.7	87.5	32.9	4.2
Unidentified unarmoured dinoflagellates	0.0	0.0	28.3	0.0	145.8	0.0	1.0
	0.0	0.0	16.3	14.7	84.7	0.0	0.0
Gonyaulax	2.2	0.0	0.0	7.5	0.0	0.0	0.0
	1.3	0.0	0.0	10.0	0.0	0.0	0.0
Peridinium	0.0	0.0	5.0	25.0	6.3	5.0	0.0
	0.0	0.0	2.5	25.0	1.6	1.6	0.2
Grand total cell no.	15.7	4721.7	160.0	100.0	337.4	111.5	33.4
	14.8	3563.3	364.9	176.8	200.8	282.6	30.6

STA. CT 109 Sampling Date	1968 Aug. 23	Sept. 22	Oct. 22	Nov. 20	Dec. 17	1969 Jan. 15	Feb. 12
Total no. of diatoms	19.4 19.4	0.9 3.5	230.3 285.6	1585.7 1465.9	4.0 3.6	0.5 0.9	5.1 3.9
Chaetoceros	7.7 7.7	0.0 1.3	177.5 222.9	9.4 6.7	0.0 0.0	0.0 0.0	0.0 0.4
Detonula	0.0 0.0	0.0 0.1	0.3 0.1	0.0 0.9	0.0 0.0	0.0 0.0	0.0 0.0
Leptocylindrus	0.0 0.0	0.0 0.0	1.4 1.2	1.8 0.0	0.0 0.0	0.1 0.0	0.0 0.0
Navicula	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Nitzschia	1.0 1.0	0.0 0.0	29.0 34.9	1532.6 1431.5	1.5 2.3	0.5 0.6	0.0 0.2
Skeletonema	5.1 5.1	0.0 1.0	1.0 1.6	6.8 4.7	2.5 0.9	0.0 0.0	0.0 0.0
Thalassiosira	0.5 0.5	0.9 0.2	15.0 17.0	11.1 10.7	0.0 0.2	0.0 0.0	2.3 1.5
Total no. of dinoflagellates	4.1 4.1	0.9 0.8	2.0 2.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Unidentified unarmoured dinoflagellates	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Gonyaulax	2.6 2.6	0.0 0.5	1.0 1.1	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Peridinium	1.5 1.5	0.9 0.3	0.3 0.5	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Grand total cell no.	23.5 23.5	1.8 4.7	233.3 288.7	1586.6 1467.3	4.0 3.6	0.5 0.9	5.1 3.9

STA. CT 109 Sampling Date	1969 Mar. 17	Apr. 17	May 14	June 14	July 1	July 26	Aug. 13
Total no. of diatoms	10.0	2024.2	470.3	330.0	99.9	56.7	32.7
	11.8	2984.4	247.0	202.7	126.3	84.6	26.7
Chaetoceros	0.0	162.6	237.6	0.0	5.0	0.0	27.6
	0.3	142.9	123.9	0.0	5.4	1.0	15.5
Detonula	0.0	1621.3	0.0	0.0	0.0	0.0	0.0
	0.0	2357.5	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	0.0	0.0	0.0	3.8	0.0
	0.0	25.0	0.0	0.0	8.4	2.2	0.0
Navicula	4.7	8.8	0.0	7.5	28.3	0.0	1.8
	5.2	4.1	1.1	4.1	12.5	0.0	2.2
Nitzschia	1.9	37.5	121.3	290.0	33.3	3.8	0.0
	2.6	95.1	64.9	151.0	42.9	4.3	1.0
Skeletonema	0.0	91.3	0.0	0.0	0.0	0.0	0.0
	0.2	100.0	0.0	0.0	0.0	1.2	0.0
Thalassiosira	2.2	78.8	103.8	7.5	0.0	0.0	0.0
	1.8	235.4	52.9	27.6	1.3	0.7	0.7
Total no. of dinoflagellates	2.5	7.6	37.6	125.0	116.7	37.5	5.1
	1.8	5.7	39.8	68.9	133.4	35.3	6.5
Unidentified unarmoured dinoflagellates	0.0	0.0	33.8	82.5	111.7	0.0	0.0
	0.0	0.0	36.3	40.8	135.9	0.0	0.0
Gonyaulax	2.2	3.8	0.0	0.0	0.0	0.0	0.0
	1.7	3.8	0.0	0.0	0.0	0.0	0.0
Peridinium	0.0	3.8	3.8	0.0	5.0	32.5	0.0
	0.1	1.9	3.5	4.2	2.5	23.0	0.5
Grand total cell no.	12.8	2031.8	507.9	455.0	216.6	100.2	54.4
	14.1	2991.1	286.8	273.6	267.6	129.0	55.8

STA. CT 8 Sampling Date	1968 Aug. 23	Sept. 22	Oct. 22	Nov. 20	Dec. 17	1969 Jan. 15	Feb. 12
Total no. of diatoms	2.3	13.5	524.8	2770.1	15.0	0.0	3.5
	1.1	13.5	889.2	2670.5	17.2	2.5	1.5
Chaetoceros	0.0	3.6	265.9	0.9	0.3	0.0	0.0
	0.0	3.6	554.8	6.4	0.5	0.4	0.0
Detonula	0.0	0.2	2.1	0.0	0.0	0.0	0.0
	0.0	0.2	1.8	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	1.8	0.9	0.3	0.0	0.4
	0.0	0.0	2.8	2.8	0.2	0.7	0.1
Navicula	0.0	0.0	0.8	0.0	0.0	0.0	0.0
	0.0	0.0	0.2	0.2	0.0	0.0	0.0
Nitzschia	0.0	0.0	217.6	2735.8	2.1	0.0	0.0
	0.0	0.0	239.9	2610.6	12.3	0.3	0.1
Skeletonema	0.0	0.0	6.4	26.5	0.0	0.0	0.0
	0.0	0.0	1.6	17.5	0.2	0.0	0.0
Thalassiosira	0.0	5.8	21.5	2.6	0.0	0.0	3.1
	0.0	5.8	50.6	19.7	0.4	0.5	1.1
Total no. of dinoflagellates	4.9	12.9	3.7	0.0	0.0	0.0	0.0
	2.8	12.9	16.4	0.5	1.0	0.1	0.0
Unidentified unarmoured dinoflagellates	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gonyaulax	4.1	1.7	0.8	0.0	0.0	0.0	0.0
	2.3	1.7	7.6	0.0	0.0	0.0	0.0
Peridinium	0.5	2.7	0.8	0.0	0.0	0.0	0.0
	0.4	2.7	3.7	0.5	0.1	0.1	0.0
Grand total cell no.	7.2	27.9	529.0	2773.5	15.0	0.0	3.5
	3.8	27.9	912.6	2672.9	17.6	2.9	1.6

STA. CT 8 Sampling Date	1969 Mar. 18	Apr. 17	May 14	June 14	July 1	July 26	Aug. 13
Total no. of diatoms	37.9	248.1	408.4	237.7	25.1	43.8	1166.9
	36.6	1760.5	307.7	276.1	50.9	34.6	1021.0
Chaetoceros	2.8	94.3	204.0	8.8	0.0	0.0	1158.1
	2.1	78.6	125.9	2.2	0.0	0.0	1007.4
Detonula	0.0	89.3	0.0	0.0	0.0	0.0	0.0
	0.0	1368.5	0.0	0.0	0.0	0.0	0.0
Leptocylindrus	0.0	0.0	0.0	0.0	0.0	8.3	0.0
	0.0	0.0	0.0	0.0	0.0	3.0	0.0
Navicula	18.8	12.5	27.0	8.8	21.3	0.0	0.0
	17.2	7.2	14.3	9.9	21.6	0.0	0.0
Nitzschia	4.3	10.8	167.0	191.3	3.8	1.3	4.4
	5.8	52.2	155.8	232.4	11.8	3.7	2.4
Skeletonema	0.0	30.0	0.0	0.0	0.0	0.0	0.0
	0.6	55.6	0.0	0.0	0.0	0.0	2.9
Thalassiosira	0.7	6.8	0.0	12.5	0.0	0.0	0.0
	1.6	185.4	3.9	9.9	1.3	0.6	2.1
Total no. of dinoflagellates	0.7	3.4	27.0	20.1	87.6	645.1	11.9
	1.8	6.4	23.1	46.1	217.8	952.7	14.6
Unidentified unarmoured dinoflagellates	0.0	0.0	27.0	3.8	83.8	590.9	0.0
	0.0	0.0	20.2	30.2	215.6	899.1	0.0
Gonyaulax	0.7	0.8	0.0	0.0	0.0	0.0	0.0
	1.6	1.4	0.0	4.1	0.0	0.0	0.0
Peridinium	0.0	2.6	0.0	16.3	0.0	30.4	1.9
	0.1	5.0	2.9	7.7	1.3	35.1	6.1
Grand total cell no.	39.8	251.5	438.0	257.8	112.6	690.2	1189.4
	39.5	1771.0	331.5	327.9	269.9	989.7	1048.9